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STUDIES ON POTATO VIRUS DISEASES

V. INSECT TRANSMISSION OF POTATO LEAF-ROLL

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(With Plates X–XII.)

CONTENTS.

1. INTRODUCTION.

IN many plant viruses there appears to exist an affinity between the virus and one particular insect which alone can disseminate it. This is illustrated by "Streak" of maize which is only transmitted by a certain species of leafhopper (7), and the "Yellows" disease of asters (2) where the same is true. It may be suggested that some such affinity exists between the virus of leaf-roll of potato and the aphis *M. persicae*, but evidence for this is not yet forthcoming. That it is the only carrier of leaf-roll is probably

not the case, and indeed other potato virus workers claim to have infected healthy plants by means of other widely differing insects (1, 4). There seems little doubt, however, in view of the evidence presented in the ensuing publication that *M. persicae* is the most efficient transmitter of leaf-roll, at all events under glasshouse conditions. The first object, then, of these studies, which was to discover an efficient insect vector of leaf-roll, may fairly be said to have been achieved, and the evidence for this is presented in the first half of this communication. The remainder of the paper is devoted to an account of some preliminary attempts to throw light upon the relations existing between virus and insect. The writer's thanks are due to Miss F. E. Hawkes for her kind assistance with the care of plants, and to Messrs F. Laing and F. V. Theobald for identifying aphides used in connection with this work.

2. MATERIAL AND METHODS.

Except for one experiment carried out in 1926 at the University of Manchester, all the work detailed in this communication was performed in the insect-proof glasshouse of the Potato Virus Research Station at Cambridge. The healthy "seed" used was partly from the writer's own stock grown for 3–4 years under insect-proof conditions and partly a number of tubers kindly given by Dr R. N. Salaman from his own stock of tested plants. The leaf-roll "seed" used for the infection of the insects was again derived partly from the writer's own stock which had been grown under insect-proof conditions for a number of years, and partly from leaf-roll tubers kindly supplied by Prof. Murphy of Dublin. The insects used, other than aphides, were collected from nettles in the vicinity of Cambridge; the aphides *M. gei* were collected from Iris sp. and the aphis *M. persicae* was from a stock, which the writer has had breeding for the past three years upon Cruciferae and other non-Solanaceous hosts.

Two methods of sprout infection were used, the first consisted in placing the sprouted half tuber in an ordinary 2 lb. glass jam jar, introducing the aphides on to the sprouts, and enclosing the top with a fine muslin cover held in place by a rubber band. The aphides failed to flourish under these conditions, and the method was abandoned for the following which proved satisfactory. The half tuber was planted in a 5 in. pot with the sprouts appearing above the soil, the aphides were then colonised upon the sprouts, and the whole covered with a glass lamp-chimney of the "hurricane" lamp type, the lower rim of the glass chimney afforded support when pushed into the ground, while a muslin cover held in place by a rubber ring round the upper rim of the glass

rendered the whole insect-proof. When the requisite time for infection had elapsed, the sprouts were cleared of aphides and the plant allowed to grow to maturity. This method gave the necessary temperature and humidity for the aphis, and a rapidly growing shoot in good condition for infection.

No sprout infection experiments of any kind were performed without half-tuber controls, and where infections were made on the growing plant either a tuber from the same parent or a cutting were grown as controls. Where no mention of the control plants is made it is implied that they remained healthy.

3. INFECTION TESTS IN 1927 WITH DIFFERENT POTATO INSECTS.

The following insects were used in the 1927 experiments:

HEMIPTERA.

HETEROPTERA.

Capsidae. *Calocoris bipunctatus* Fab. (*norvegicus*).
Lygus pabulinus Linn.

HOMOPTERA.

Jassidae (Leaf-hoppers). *Eupteryx auratus* Liv.
Chlorita viridula Fall.

Aphididae. *Macrosiphum gei* Koch.
Myzus persicae Sulz.

COLEOPTERA.

Psylliodes affinis Pk. Potato Flea Beetle.

HEMIPTERA. HETEROPTERA, Capsidae.

Calocoris bipunctatus. The infection experiments with this insect were divided into four series, each series consisting of six sprouted half tubers, with half tuber controls. The source of infection was a number of leaf-roll potatoes var. British Queen, and the half tubers used were known healthy Arran Victory. The capsids were collected in the vicinity of Cambridge from nettles only, and both adult and larval forms were used. Details, and results, of the inoculation tests with this insect are given in Table I.

A larger number of capsids per half tuber was found to be unsatisfactory, as the feeding punctures tended to destroy the sprouts.

Some experiments on the same lines as those carried out in the mosaic work (5) were also performed with this insect and leaf-roll. The salivary

Table I.

	Series 1	*Series* 2	*Series* 3	*Series* 4
Source of infection	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen
Time on source	7 days	10 days	14 days	21 days
Time on exp. half tuber ...	7 days	17 days	14 days	12 days
Mean daily temp.	58° F.	69° F.	64° F.	68° F.
No. of capsids per half tuber	1	2	2	3
No. of capsids still alive at end of exp.	6	12	11	16
No. of half tubers inoculated	6	6	6	6
No. of plants infected ...	0	0	0	0

glands of a number of this species of capsid which had fed on a leaf-roll plant were extracted and inserted into the sprouts of half tubers. These all gave healthy plants. A further experiment was performed in which the faeces of capsids, bred on leaf-roll plants were inoculated into sprouting half tubers, this experiment was also negative.

Lygus pabulinus. Four similar series of sprout inoculations were performed with another species of capsid bug, *i.e. Lygus pabulinus* also from nettles (Table II).

Table II.

	Series 1	*Series* 2	*Series* 3	*Series* 4
Source of infection	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen
Time on source	7 days	14 days	17 days	21 days
Time on exp. half tuber ...	10 days	14 days	14 days	11 days
Mean daily temp.	59° F.	64° F.	64° F.	68° F.
No. of capsids per half tuber	1	1	2	4
No. of capsids still alive at end of exp.	6	5	10	20
No. of half tubers inoculated	6	6	6	6
No. of plants infected ...	0	0	0	0

HOMOPTERA. Jassidae (Leaf-hoppers).

Eupteryx auratus, Chlorita viridula. Two series of sprout inoculations were performed with the first of these, and one series with the second. It was necessary to use half tubers which had produced large leafy shoots before the hoppers could be induced to feed (Table III).

Aphididae.

Aphididae. Two series of sprout inoculations were carried out with the aphis *Macrosiphum gei*, collected from Iris and other non-solanaceous host plants (Table IV).

Table III.

	Series 1 (*E. auratus*)	*Series* 2 (*E. auratus*)	*Series* 3 (*C. viridula*)
Source of infection	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen
Time on source	9 days	14 days	10 days
Time on exp. half tuber	10 days	14 days	10 days
Mean daily temp.	60° F.	60° F.	60° F.
No. of leaf-hoppers per half tuber	3–4	4	4
No. of half tubers inoculated ...	6	6	6
No. of plants infected	0	0	0

Table IV.

	Series 1	*Series* 2
Source of infection	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen
Time on source	Bred on leaf-roll plant	Bred on leaf-roll plant
Time on exp. half tuber ...	16 days	23 days
Mean daily temp.	60° F.	65° F.
No. of aphides per half tuber	12–18	50–100
No. of half tubers inoculated	6	6
No. of plants infected ...	0	0

The second species of aphis used was the small green aphis *Myzus persicae* Sulz. Six series of experiments were carried out with this insect—four sets of six sprouted half tubers, colonised with adult aphides, from leaf-roll British Queen, and two sets of six sprouted half tubers colonised with larval forms only, also from leaf-roll British Queen. Details of the experiments and the results achieved are set out in Table V.

Table V.

	Adult aphides				Larval forms	
	Series 1	*Series* 2	*Series* 3	*Series* 4	*Series* 5	*Series* 6
Source of infection	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen
Time on source	Bred on l.-r. plant	Bred on l.-r. plant	Bred on l.-r. plant	Bred on l.-r. plant	Bred on l.-r. plant	Bred on l.-r. plant
Time on exp. half tuber	23 days	21 days	20 days	21 days	18 days	18 days
Mean daily temp.	63° F.	62° F.	63° F.	60° F.	63° F.	63° F.
No. of aphides per half tuber	12–18	12–18	12–18	12–18	12–18	12–18
No. of half tubers inoculated	6	6	6	6	6	6
No. of plants infected	6	6	6	6	4	4

Out of the 24 half tubers inoculated with adult infective aphides, 24 infected plants were produced; and out of 12 half tubers inoculated with the larval forms, 10 infected plants were produced. This shows the larval forms to be efficient carriers of leaf-roll, provided they have fed on a leaf-roll plant sufficiently long to pick up the virus. In the above experiments symptoms developed in an average period of 32 days from the first date of infection.

COLEOPTERA.

Psylliodes affinis. Potato Flea Beetle. One series only of experiments was carried out with this insect. With this beetle as with the leaf-hopper it was necessary to use half tubers with large leafy sprouts, in order that the insect might be induced to feed (Table VI).

Table VI.

Source of infection	Leaf-roll Brit. Queen
Time on source	24 days
Time on exp. half tuber ...	12 days
Mean daily temp.	68° F.
No. of beetles per half tuber	2
Results	All plants healthy

It is realised that such a limited experiment as the above can only be regarded as a preliminary test of the leaf-roll carrying powers of this insect.

It will be seen from the foregoing tables of sprout infections, that out of the seven different insects tested only one, the aphis *M. persicae*, gave positive results. This aphis, as later experiments will show, has proved itself to be a most efficient carrier of leaf-roll. The progeny of the plants arising from the experimental half tubers used in all these tests were grown the following year (1928). All produced healthy plants with the exception of the thirty-four successful inoculations with *M. persicae* which gave leaf-roll plants (Plate X, fig. 2).

Haulm Infection Experiments in 1927 *with* Eupteryx auratus.

As it was found difficult to induce this leaf-hopper to live satisfactorily upon the sprouts of the tuber, three series of infections of the haulm were carried out. In the second series of experiments, the leaf-hoppers used were the progeny of a number of leaf-hoppers which had been allowed to breed on a leaf-roll plant. These insects, during their lifetime therefore, had fed only upon leaf-roll potato foliage (Table VII).

Table VII.

	Series 1	*Series* 2	*Series* 3
Source of infection ...	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen	Leaf-roll Brit. Queen
Time on source	14 days	Bred	Bred
Time on exp. plant ...	18 days	16 days	13 days
Mean daily temp. ...	65° F.	67° F.	Out of doors insectary
No. of plants inoculated	6	6	6
No. of plants infected ...	0	0	0

All these plants remained healthy throughout 1927. The progeny were grown in the field during 1928. On July 26th, 1928, it was noticed that three plants showed leaf-roll; there is therefore a possibility that the leaf-hopper had infected the parent plants the year before. The writer however is more inclined to the view that the disease resulted from external infection by *M. persicae* in the field during the summer of 1928, especially as the three plants infected were not all the progeny of one parent but isolated plants.

4. INFECTION TESTS IN 1928 WITH *MYZUS PERSICAE* SULZ.

Following upon the successful sprout inoculations with this insect in 1927, further inoculation tests were performed in 1928. The first four series consisted each of twelve sprouted half tubers, var. Arran Victory, with half tuber controls; these were colonised in glass jars and later cleared of aphides and potted up. The fifth series, consisting of six half tubers, var. President, were infected by means of the improved method already described, where the half tuber is planted in a pot and covered with a glass lamp chimney. This method was adopted for all further sprout inoculations with insects. In the first two series of sprout infections the immature forms only of the aphis *M. persicae* were used. The sprouts were infected in the glasshouse on February 29th, on March 20th the sprouts were cleared of aphides and potted up. Symptoms of leaf-roll appeared on April 16th, 36 days after the first infection of the sprouts with the virus-carrying aphides. By the end of April 20 plants out of the 24, arising from the experimental half tubers, had developed leaf-roll, the other four remained healthy. On March 21st the two further series of experiments with Arran Victory were performed; this time adult aphides were used and were allowed to feed for 28 days before the sprouts were cleared. The half tubers were planted on April 27th; nine days later one of the plants, now a few inches above ground, showed slight symptoms of leaf-roll. By May 16th all 24 plants had developed leaf-roll (see Plate X, fig. 4). The

fifth series, six half tubers var. President, were colonised with aphides on April 19th, these were cleared of aphides 14 days later, May 3rd, by May 25th all six plants were leaf-rolled (Plate XI, figs. 1–3). In one case the plant developed symptoms soon after its appearance above ground. Details of these sprout inoculations are given in Table VIII.

Table VIII.

1928 *Sprout Inoculations with* Myzus persicae.

	Series 1	*Series* 2	*Series* 3	*Series* 4	*Series* 5
Commencement of experiment	Feb. 29th	Feb. 29th	March 21st	March 21st	April 19th
Date of appearance of first symptoms	April 16th	April 20th	May 6th	May 9th	May 20th
Variety of half tubers	Arran Victory	Arran Victory	Arran Victory	Arran Victory	President
Source of infection	Leaf-roll British Queen in each case				
Time on source	Bred	Bred	Bred	Bred	Bred
Time on exp. half tuber	20 days	20 days	28 days	28 days	14 days
Mean daily temperature	62° F.	62° F.	65° F.	65° F.	65° F.
No. of aphides per half tuber	6	6	12–18	12–18	12–18
Description of aphides used	larvae	larvae	adults	adults	adults
No. of half tubers used	12	12	12	12	6
No. of plants infected with leaf-roll	10	10	12	12	6

1928 *Haulm Inoculations with* Myzus persicae.

Two series of infections of the haulms of young growing plants were carried out with *M. persicae* infected with the virus of leaf-roll. The experiments were performed under canvas cages in one of the compartments of the insect-proof glasshouse. The first series consisted of six young Arran Victory plants, which were colonised with the infective aphides on March 6th. On April 11th the first symptoms of leaf-roll appeared, and by the end of the month five out of the six plants had developed the disease (see Plate XI, fig. 6). The sixth plant which was poorly colonised with the aphis remained healthy.

In the second series six young plants var. President were used, and colonised with aphides on April 2nd. The first symptoms of leaf-roll appeared on April 30th, by the middle of May all six plants had developed leaf-roll. A third experiment was performed in the open air insectary as follows: a leaf-roll British Queen plant on which a number of *M. persicae* were feeding, was placed near to, but not touching, six young Arran

Victory plants of known healthy stock. No aphides were transferred from the leaf-roll plant but were allowed to breed untouched. After about six weeks one of the healthy Arran Victory plants developed leaf-roll to be followed shortly after by a second, showing that infection had been carried by migration of the aphis from the leaf-rolled plant. The experiment was then discontinued. The large number of positive infections obtained under controlled conditions by means of *Myzus persicae*, detailed in the foregoing experiments, are sufficient in the writer's opinion to incriminate this aphis as an efficient vehicle for the dissemination of leaf-roll.

Although entirely negative results were obtained in the inoculation experiments with the other potato feeding insects, it is unwise to deduce on that account that such insects are unable under any circumstances to disseminate leaf-roll. Suffice it to say that so far they have not done so, under conditions giving positive infections with *Myzus persicae*. Other potato virus workers, Murphy (4) and Elze (1), are of the opinion that some at least of these insects are capable of transmitting leaf-roll.

The development of leaf-roll in the variety Arran Victory after inoculation by *M. persicae*, which has been closely studied by the writer, exhibits a fairly consistent sequence of symptoms. The first signs of the disease (primary leaf-roll) appear about thirty days after the date of insect colonisation; they consist first of a general pallor of the upper and youngest leaves followed later by a rolling starting from the base and usually accompanied by a well-marked pigmentation, brownish-black in colour, which is characteristic of leaf-roll in this variety. Under glasshouse conditions, this primary leaf-roll rapidly passes into the secondary form in which the lower leaves show a marked interveinal pallor, becoming leathery and harsh to the touch. They then begin to roll, usually developing at the same time the brownish-black pigmentation which may occupy the whole base of the leaf, as shown in Plate XI, fig. 5. The whole plant is stunted, often presenting a purplish tint, and in a bad case every leaf may be rolled. In Arran Victory a tendency to form aerial tubers is often associated with leaf-roll. Half tubers inoculated by means of *M. persicae* at the end of February and the beginning of March produced plants with well marked secondary leaf-roll within two months of that date.

These plants differed in no degree of severity from the progeny of plants infected with leaf-roll by the aphis in the preceding year (Plate X, fig. 2). In the glasshouse the half tubers infected by *M. persicae* with leaf-roll produced plants which averaged from 9–12 in. in height and gave a negligible yield of tubers, while the control half tubers grown under the

same conditions but without insect inoculation produced plants which averaged 5 ft. in height and gave a good crop of tubers.

Sufficient evidence of the power of this aphis to transmit leaf-roll having been offered, some further investigations into the part played by this insect in the dissemination of leaf-roll are described.

5. NON-TRANSMISSION OF THE LEAF-ROLL VIRUS TO THE PROGENY OF THE APHIS.

Experiments to determine this somewhat important point were carried out during the years 1927–1928. In 1927 the experiments were planned as follows: 25 mature viviparous female *M. persicae*, which had been bred on a leaf-roll British Queen plant, were placed each one in an empty glass-bottomed pill box. As each parthenogenetically produced young appeared, it was picked up with a fine camel's hair brush and placed upon a sprouted half tuber var. Arran Victory with half tuber control. All the young produced by each female aphis were kept together on their respective half tubers. When sufficient young aphides, 6–9, had been produced, the mother aphis was placed on a sprouted half tuber in order to demonstrate that she herself was infective. This precaution was taken because the writer is of the opinion that the virus-carrying powers may differ in individual aphides of this species. By the pill box method described above (first used by Murphy in Dublin (4)) all fear of contamination of the young aphis from other sources is eliminated. Thus far the experiment consists, then, of 25 sprouted half tubers each with one adult female aphis, with 25 half tuber controls, and 25 half tubers each with 6–9 young aphides and 25 controls, each set of young corresponding to one female aphis. When the young of the first generation were mature, they in their turn were placed in pill boxes, and the young transferred to a further 25 sprouted half tubers with controls. This was to investigate the possibility of inheritance of the virus to the second generation. The 150 half tubers constituting the whole experiment were grown in the insect-proof glasshouse under identical conditions, all remained healthy with the exception of 5 (20 per cent.) of the plants produced by those half tubers which had been colonised with the original virus-carrying females. This experiment was therefore negative as regards the inheritance of the virus by the aphis progeny; one point however arises, *i.e.* that one aphis is capable of infecting a plant with leaf-roll. The facts that *M. persicae* tends to die easily when away from any food plant for more than 24 hours, and also that under starvation conditions it does not reproduce readily, rendered the experiment somewhat difficult to perform owing to

the necessity of replacing the adults which constantly died without reproducing. In 1928 the work was simplified, ten half tubers only were colonised with the young from adult females placed in glass boxes as before, and the second generation was not considered. This gave a total of 20 experimental plants and 20 controls; of the ten plants arising from the half tubers colonised with the adult aphides five were infected with leaf-roll, while the ten infected with the young aphides remained healthy. So far as negative evidence can be conclusive, these experiments carried out over two years, indicate that the virus is not inherited by the progeny of an infective aphis. This conclusion is in agreement with the findings of workers on other insect-carried plant viruses, and the writer is not aware of an authoritative case of such inheritance other than that quoted in the work of McClintock and Smith (3) on spinach blight.

6. EFFECT ON HEALTHY POTATO PLANTS OF VARYING DOSES OF VIRUS.

The object of this experiment was to ascertain whether the *number* of infective aphides attacking a plant was of importance, and whether the plant would react differently to the varying amounts of virus received in consequence. Sprouted half tubers were used (var. Arran Victory) and six series of experiments were performed, each one consisting of four inoculation tests, *i.e.* 2, 6, 12 and 18 aphides respectively per half tuber. The aphides used for each experiment had all been bred on the same leaf-roll plant, and were as far as possible uniform as to size, age, etc. Details of the experiments are given in the table. The results show that the incidence of infection is greatest among those plants colonised with 12 and 18 aphides. Nevertheless infection was produced among the plants colonised with two aphides only, and when achieved the disease differed in no way from that produced by 18 aphides. This is in agreement with Storey's findings with streak disease of maize (7). The small incidence of infection among the plants treated with two and six aphides may be explained partly by the fact that *M. persicae* is very sensitive to environment, and if conditions of temperature and humidity are not suitable, it tends to leave its food plant and wander away, and in an experiment of this kind obviously the aphides could not be replaced without risk of nullifying the experiment. Another possible explanation is variability in the infective power of individual aphides (Table IX).

Table IX.

	Experiment 1				*Experiment* 4			
	2 aphides	6 aphides	12 aphides	18 aphides	2 aphides	6 aphides	12 aphides	18 aphides
Date of experiment	May 14	May 14	May 14	May 14	May 21	May 21	May 21	May 21
First appearance of symptoms	Healthy	Healthy	June 12	June 12	June 21	June 21	June 18	June 15
Incubation period in plants	—	—	29 days	29 days	31 days	31 days	28 days	25 days
	Experiment 2				*Experiment* 5			
Date of experiment	May 16	May 16	May 16	May 16	June 15	June 15	June 15	June 15
First appearance of symptoms	June 19	June 18	June 18	June 12	Healthy	Healthy	July 12	July 12
Incubation period in plants	34 days	33 days	33 days	27 days	Healthy	Healthy	27 days	27 days
	Experiment 3				*Experiment* 6			
Date of experiment	May 18	May 18	May 18	May 18	July 1	July 1	July 1	July 1
First appearance of symptoms	Healthy	Healthy	June 18	June 20	Healthy	Healthy	Healthy	Aug. 5
Incubation period in plants	—	—	31 days	33 days	Healthy	Healthy	Healthy	35 days

7. COLONISATION OF INFECTIVE APHIDES UPON IMMUNE PLANTS FOR VARYING PERIODS BEFORE THEIR SUBSEQUENT TRANSFER TO HEALTHY POTATOES.

Experiments were performed to determine the effect produced on the infective power of *M. persicae* by allowing the virus-carrying aphides to feed upon immune non-solanaceous plants before transferring them to healthy potatoes. It was considered possible that prolonged feeding upon immune plants might have the effect of clearing the virus from the body of the aphis and thus render it non-inoculative, especially if the insect be a mechanical carrier of the virus. The immune plant selected for this work was the cabbage and the experiments were performed as follows: aphides which had been bred upon leaf-roll British Queen potato plants were allowed to feed on cabbage for 24, 48, and 72 hours, and 7 days respectively; they were then transferred, each lot, to three sprouted half tubers var. Arran Victory. At the same time a number of the same stock of infective aphides were transferred directly to three sprouted half tubers var. Arran Victory, to demonstrate that this particular lot of aphides was actually carrying the virus. The results were as follows: the three half tubers colonised with aphis direct from the leaf-roll plant produced plants which developed the disease 22 days later; the three half tubers colonised with aphis after 24 hours on cabbage gave rise to diseased plants 23 days later, those with aphis 48 and 72 hours on cabbage, 22 and 18 days later

respectively, and those with aphis 7 days on cabbage, 26 days later (Plate XII, Fig. 1). The experiment was then repeated using a different set of *M. persicae* from another leaf-roll plant, and omitting the direct infection of potato and the 72-hour period on cabbage. The results were the same as in the preceding experiment—two out of the three plants in the 24 hours experiment developed leaf-roll in 21 days, the third plant remained healthy. The 48 hours and 7 days period experiments all developed the disease in 22 and 20 days respectively. From these results it will be seen that feeding upon a non-susceptible plant host has no effect upon the infective power of the aphis. It seems probable, but this has not yet been proved, that *Myzus persicae* once infected remains so for the rest of its life. With so prolific an insect as the aphis it is a difficult matter to keep individual insects under observation for more than a week, rapid multiplication rendering it difficult to trace the original insects used. In order therefore to feed infective aphides upon cabbage for longer periods than a week, it would be necessary to pick off the young each day as born, and where large numbers of aphides are employed this is no small undertaking. Details of the experiment are given in Table X. An investigation into

Table X.

Colonisation of infective aphides upon immune plants before their transfer to healthy potatoes.

	Direct transfer to healthy potatoes	24 hours on cabbage	48 hours on cabbage	72 hours on cabbage	7 days on cabbage
		Experiment 1.			
Date of experiment	May 30th	May 30th	May 30th	June 18th	May 30th
Date of first symptoms	June 19th	June 22nd	June 21st	July 6th	June 26th
No. of half tubers inoculated	3	3	3	3	3
No. of plants infected with leaf-roll	3	3	3	3	3
		Experiment 2.			
Date of experiment	—	June 16th	June 16th	—	June 18th
Date of first symptoms	—	July 6th	July 7th	—	July 9th
No. of half tubers inoculated	—	3	3	—	3
No. of plants infected with leaf-roll	—	2	3	—	3

the effect of starvation upon the transmitting powers of *M. persicae* was also carried out. It was found that aphides remained infective after

4–5 days' starvation, about the maximum period without food to which this aphis can be subjected.

8. SEPARATION BY *M. PERSICAE* OF LEAF-ROLL FROM A COMBINATION OF TWO VIRUSES.

Some preliminary experiments were performed to determine the virus-carrying powers of *M. persicae* after feeding upon a potato plant affected with a combination of two viruses of which one was leaf-roll. Two sets of experiments were carried out: (*a*) with a leaf-roll streak combination, (*b*) with leaf-roll mosaic.

In the first case the leaf-roll streak combination was obtained as follows: an Up-to-Date plant which was known to be "carrying" streak, and which had been tested by grafting on to Arran Victory, a variety very susceptible to streak, was infected early in the season with leaf-roll by means of *M. persicae*. It is perhaps worthy of mention that infection of the Up-to-Date plant with leaf-roll seemed to cause the appearance of streak symptoms hitherto suppressed (Plate XII, fig. 2). This plant, then, showing plainly the symptoms of both diseases was colonised with *M. persicae*, which later were transferred in the usual way to six sprouted half tubers var. Arran Victory, and three half tubers var. President. Symptoms of leaf-roll developed in two Arran Victory plants in 31 days and in two more Arran Victory plants in 37 days. The three President and the two remaining Arran Victory plants remained healthy. Although both President and Arran Victory are exceedingly susceptible to streak and both varieties had actually been infected from this same plant by grafting, no symptoms of this disease appeared, thereby further confirming the writer's inability to induce any insect to transmit streak as such from diseased to healthy plants. The results of this experiment indicate that *M. persicae* will pick up the leaf-roll virus from a combination of streak and leaf-roll and leave the streak behind, or perhaps it would be more correct to say that the plant inoculated by the aphis from the streak leaf-roll plant apparently receives only leaf-roll.

The leaf-roll mosaic combination in the second experiment was obtained by infecting a mosaic Arran Victory with leaf-roll by means of *M. persicae*. When the plant had developed leaf-roll it was colonised with *M. persicae* for transfer to healthy half tubers. In this experiment an attempt was made to discover whether the aphis was picking up both viruses; in a previous publication(5) the writer has shown that the tobacco plant is a sensitive indicator to potato mosaic carried by *M. persicae*. Aphides from the leaf-roll mosaic Arran Victory were therefore

colonised on healthy sprouted half tubers, and also upon a number of healthy tobacco seedlings (var. White Burley). The results of this experiment were as follows: the half tubers of healthy Arran Victory produced leaf-roll plants, first symptoms developing after 25 days. The tobacco plants which do not react to the leaf-roll virus developed the typical mottle disease which mosaic-bearing *M. persicae* produce on tobacco (5). This shows then that the aphis apparently picks up both viruses and that the separation of them depends more upon the plant than the insect. The above are preliminary experiments only and further work on these lines is contemplated.

9. INCUBATION PERIOD OF THE VIRUS IN THE PLANT.

The incubation period of the leaf-roll virus in the plant, counting from the first day of colonisation of the sprouted half tuber with the aphis, varies between 18–50 days, with an average period of 30 days at a mean daily temperature of 65° F. The periods of 40–50 days only occurred in those experiments where the infective aphides were allowed to remain on the unplanted half tubers for 20 days or more before the latter were potted up. Infection with leaf-roll by means of *M. persicae* is therefore usually more rapid than by means of grafting, presumably because the period of waiting for union of scion and stock is eliminated. The question of the incubation of the virus in both plant and insect is still under investigation. Experiments are in progress which are designed to show with considerable accuracy the duration of the incubation period of the virus in the plant, the incubation period, if any, of the virus in the body of the insect, and the time necessary for the insect to feed upon an infected plant in order to pick up the leaf-roll virus.

10. INFECTION OF DIFFERENT VARIETIES OF POTATO WITH LEAF-ROLL BY *M. PERSICAE*.

With a view to ascertaining whether any difference in degree of resistance to leaf-roll existed in different varieties of potatoes, inoculations with *M. persicae* were made with the following varieties: President, King Edward, Arran Chief, Kerr's Pink and Great Scot, to which may be added Arran Victory so largely used in the experimental work, and Edzell Blue which was experimentally infected in 1926. All these varieties developed leaf-roll in the current season. The tubers used in this experiment were tested healthy stock, and the infective aphides were colonised on sprouted half tubers with the usual half tuber controls. Some details

of the development of the leaf-roll symptoms in the different varieties are given.

President. The first sign of the disease in this variety is a pallor at the edges of the young leaves, later this spreads to the lower leaves which become much thickened and leathery. There is less actual rolling of the leaves in President than in Arran Victory. The whole tendency of a leaf-roll President plant is towards a stiff upright habit. Often the young leaves show a pale yellowish coloration on the upper side with an accumulation of pink pigment on the lower (Plate XI, fig. 4). The whole plant is very harsh to the touch and rattles when shaken (Plate XI, figs. 1–3).

Great Scot. Six half tubers of this variety were colonised with infective aphides on June 22nd; first signs of leaf-roll appeared on one plant on July 12th, 20 days later. By July 26th all six plants were infected. Actual rolling in this variety does not appear to be very pronounced, first symptoms appeared as a marked interveinal pallor, accompanied by a stiffness of the lower leaves, later some rolling develops (Plate XII, fig. 4).

King Edward. Six half tubers were colonised with infective aphides on July 4th, symptoms of leaf-roll developed in one plant on July 31st, 27 days later. All plants developed leaf-roll by August 14th. A general pallor first appeared on the young leaves followed by a slight rolling at the base. Pink coloration developed on the lower surface of both young and old leaves, later fairly pronounced rolling appeared on the lower leaves accompanied by the usual stiffness and leatheriness (Plate XII, fig. 5).

Arran Chief. Six half tubers infected on July 2nd produced diseased plants on July 31st, 29 days later. First symptoms appeared as a marked interveinal pallor mostly on the young leaves, later rolling developed accompanied by some pink pigmentation, the leaves becoming yellowish on the upper surface, stiff and harsh.

Kerr's Pink. Six half tubers were infected on June 28th, first symptoms appeared on August 2nd. The young leaves developed an interveinal pallor accompanied by rolling at the base with some pink pigmentation. Three plants only developed current season symptoms.

11. FEEDING POINT OF APHIS IN RELATION TO INFECTION OF THE HOST PLANT.

It has already been conclusively demonstrated that the leaf-roll virus is distributed by infective aphides feeding either upon the sprouts of the tuber, or upon the leaves of a young growing plant, although infection

may take longer to develop when the aphis feeds on the haulm. An experiment was performed to determine whether the plant was as easily infected when the aphis fed only upon the stem. Three half tubers var. President were planted when the resulting plants were five inches high, all the leaves were removed. Infective aphides were then colonised on the bare stem; the new shoots arising from the other eyes of the half tubers developed leaf-roll when about three inches high, 34 days after colonisation with the aphides.

As would perhaps be expected, this experiment shows that infection with leaf-roll is as easily brought about by aphis punctures in the stem as by punctures in sprout or leaf.

12. PASSAGE OF THE VIRUS THROUGH THE PLANT, BEFORE DEVELOPMENT OF LEAF-ROLL SYMPTOMS.

In this experiment a number of half tubers were used in which all the eyes had been removed save two, diagonally opposite each other. One shoot was colonised with infective aphides, the other was kept uninfected. Leaf-roll appeared simultaneously in both shoots showing that the virus had passed down the infected shoot and through the half tuber into the uninfected shoot before the symptoms of the disease developed.

13. DISCUSSION.

A number of potato-feeding insects mostly of the plant-sucking Hemiptera have been tested as to their capabilities for transmitting leaf-roll. The aphis *Myzus persicae* Sulz. is outstanding as a most reliable disseminator of this disease. Although tests with the other insects proved negative, much more work remains to be done with those insects before they can be regarded definitely as incapable of transmission. That *M. persicae* is a more efficient carrier of the virus can hardly be doubted, and it is probable that this insect is responsible for much of the spread of leaf-roll in the field. In the glasshouse it is possible to transmit leaf-roll to a healthy plant by means of *M. persicae* with almost the same reliability as grafting and in a shorter time. The disease first appears in its "primary" form where the young leaves are affected (Plate X, figs. 1 and 4), but this very rapidly passes, at all events under glasshouse conditions, into the "secondary" condition where the whole plant is affected (Plate X, figs. 5 and 6).

As regards the other experiments described in this paper, some points of interest have arisen. Two years' work on the question of the heredity of the leaf-roll virus by the progeny of the aphis has given negative

results, and it may fairly be concluded that the virus is not inherited; this is in accord with the findings of most workers upon insect-borne plant viruses. It has also been shown that two individuals of *M. persicae*, and in a few cases one, are capable of infecting a plant with leaf-roll, the disease thus produced differing in no degree of severity from that produced by 18 infective aphides. That the aphis is no mechanical carrier in the sense that the virus adheres merely to the external mouthparts is shown by the fact that periods up to seven days' feeding on immune plants such as cabbage are no obstacle to the subsequent infection of healthy potato plants, though this fact cannot be taken as evidence of a direct relationship between virus and insect. The whole question of such relationship, if one exists, between the aphis and the leaf-roll virus is one of great interest, but more evidence is still required on this point. That it is possible for two distinct viruses to exist together unchanged in the body of the aphis is suggested by the preliminary experiments on the transmission of combinations of leaf-roll and mosaic. By placing the aphides, after feeding upon a plant with this combination, upon other plants which react differently to the two viruses, *i.e.* tobacco and potato, it was found that the former developed mosaic and the latter leaf-roll. This appears to show that whatever virus or viruses be present in a plant they are picked up by the aphis and passed on to the next host plant, which develops the disease to which it may be most susceptible, in this case the tobacco is most susceptible to potato mosaic and the potato to leaf-roll. As to why *M. persicae* is a more efficient carrier of leaf-roll than other insects, it is difficult to say in the present state of knowledge of this subject. It may be due to the fact that leaf-roll is primarily a phloem disease, and as the writer has shown (6) *M. persicae* is much more a phloem feeder than many of the other Hemiptera, though not more than other aphides. This explanation hardly seems to be the correct one in view of the facts that it is not possible to inoculate a potato, by means of the needle, with leaf-roll but it is with mosaic, and if it were a question, merely, of reaching the phloem with the virus, that could surely be accomplished with the needle. It may be that passage of the leaf-roll virus through the body of the aphis increases its infective power or attunes it in some way to reception by the plant. It has been shown (5) that the virus of potato mosaic transmitted to tobacco by the aphis produces a disease in which the symptoms differ from those exhibited by the disease arising from needle inoculations into tobacco with the same virus. This may be a case where the virus has changed slightly in the body of the aphis, or again it may be that difference in symptoms arises from the difference in the mode of

inoculation. So far as the experiments on aphis infection of varieties go, no very marked difference in degree of resistance to leaf-roll was apparent, all the varieties tested developing leaf-roll within the current season. The incubation period of the virus in the plant is usually about 30 days, counting from the first day of colonisation with the infective aphides. As with most plant viruses a condition of rapid growth and movement is necessary for infection with leaf-roll, and it was found increasingly difficult as the season advanced to obtain successful inoculations with the aphides.

The actual feeding point of the aphis on the plant makes little difference to ultimate infection; it is possible for the aphis to disseminate the leaf-roll virus by feeding upon the sprouts of the tuber, the haulm of the plant or upon the stem itself.

14. SUMMARY.

1. Inoculation experiments with seven different species of insects carried out in 1927 gave negative results, except in the case of the aphis *Myzus persicae* Sulz. which gave a high percentage of positive infections.

2. Further inoculation tests with this aphis in 1928 proved it to be an efficient carrier of the leaf-roll virus.

3. Experiments carried out during two years on the question of the inheritance of the leaf-roll virus by the progeny of infective aphides proved negative. It is therefore assumed that the virus is not hereditary in the offspring of the aphis.

4. One or two virus-bearing aphides are capable of infecting a healthy potato plant with leaf-roll. Such infection when achieved differed in no degree of severity from that induced by 18 aphides. The incidence of infection was greatest among plants colonised with groups of 18 infective aphides.

5. Colonisation of virus-bearing aphides upon non-solanaceous hosts such as cabbage for periods varying from 24 hours to 7 days did not affect the subsequent infective power of such aphides.

6. *M. persicae*, when colonised upon plants affected with combinations of viruses of which leaf-roll was one constituent, transmitted only leaf-roll to healthy potatoes. The combinations used were leaf-roll and streak, and leaf-roll and mosaic. In the latter case the aphis was shown, by its infection of tobacco with a mottling disease, to be picking up both viruses of which only leaf-roll developed in the potato.

7. The incubation period of the leaf-roll virus in the plant averaged

about 30 days under glasshouse conditions. In some cases the disease was found to develop in 18–20 days.

8. Seven varieties of potato have been infected with leaf-roll by means of *M. persicae*. There existed no apparent difference in degree of resistance to leaf-roll.

9. The leaf-roll virus can be disseminated by the feeding of *M. persicae*, either on the sprouts of the tuber, on the leaves and shoots of the growing plant, or on the stem alone.

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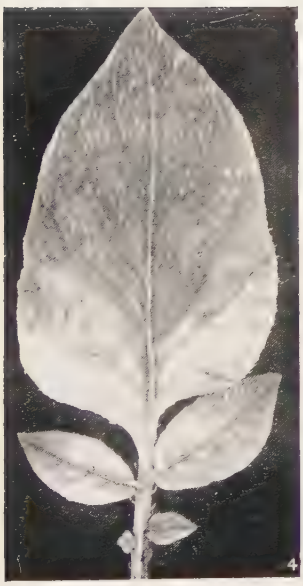

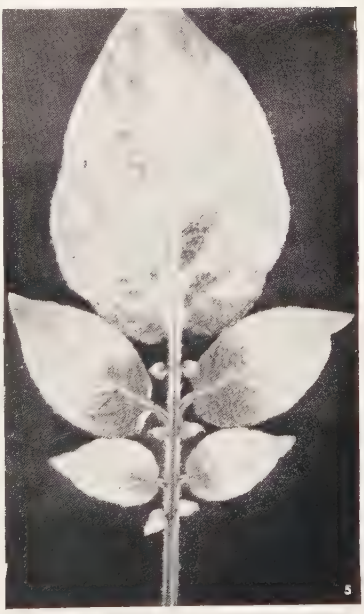

SMITH.—Studies on Potato Virus Diseases (pp. 209–229).

EXPLANATION OF PLATES X—XII.

PLATE X.

Fig. 1. Arran Victory plant arising from sprouted half tuber infected by *M. persicae* late in the season of 1927, showing "primary" leaf-roll.

Fig. 2. Progeny of the plant shown in Fig. 1 grown in 1928, exhibits marked secondary leaf-roll.

Fig. 3. Healthy controls to plants shown in Figs. 4, 5, 6.

Fig. 4. Three plants of Arran Victory, arising from sprouted half tubers infected by *M. persicae*, February 1928, showing "primary" leaf-roll.

Figs. 5, 6. Two of the three plants shown in Fig. 4 photographed six weeks later, now showing "secondary" leaf-roll. The 24 plants, of which these are two, were as severely affected in the current season as the plant shown in Fig. 2 which was infected the previous year.

PLATE XI.

Figs. 1, 2, 3. President plants, showing advanced leaf-roll, arising from sprouted half tubers infected by *M. persicae*, current season infections. Note the stiff, upright habit characteristic of this variety.

Fig. 4. Leaves of leaf-roll potato, var. President, showing the presence of pale yellow coloration at the leaf bases on the upper surface. This coloration is typical of leaf-roll in President. The lower surface of the leaves often exhibits a pink pigmentation. Compare Fig. 5.

Fig. 5. Leaves of leaf-roll potato, var. Arran Victory, showing the brownish-black pigment at the leaf bases on the upper surface. This pigmentation is characteristic of leaf-roll in Arran Victory. Compare Fig. 4. (The leaves illustrated in these two figures were photographed between two glass plates to flatten out the rolling.)

Fig. 6. Current season infection of Arran Victory with leaf-roll. This plant was one of a series infected by *M. persicae* feeding on the haulm, instead of the sprouted half tuber.

PLATE XII.

Fig. 1. Current season infection of Arran Victory with leaf-roll. The plant on the left was infected by virus-carrying aphides (*M. persicae*) after they had fed for 72 hours on cabbage, that on the right in the same way after 7 days on cabbage.

Fig. 2. Leaf-roll streak Up-to-Date plant used in experimental infections. Note the rolling of the leaves and the killing of the shoots by streak (*x*).

Fig. 3. Infection of different potato varieties with leaf-roll by *M. persicae*. Edzell Blue.

Fig. 4. Infection of different potato varieties with leaf-roll by *M. persicae*. Great Scot.

Fig. 5. Infection of different potato varieties with leaf-roll by *M. persicae*. King Edward.

Photographs by C. W. Williamson.

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INVESTIGATION OF HOP MOSAIC DISEASE IN THE FIELD

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(With 2 Text-figures.)

During the years 1923–5 field observations of Hop Mosaic were carried out to study more fully the symptoms of the disease, to follow more completely its spread under commercial conditions, and at the same time to determine how far the disease can be controlled by the prompt grubbing of diseased plants. These observations, made in a number of gardens in various parts of Kent, extended over the three years, and the following is a summary of some of the results that may be of interest on the practical side.

Infectious Nature of the Disease.

In the garden *B*, where the disease was rampant, a study of the position of diseased hills occurring during the three years, shows that although plants far removed from other diseased plants may develop the disease, yet in general the disease clearly spreads from areas which have been occupied by such plants.

The history of this garden was interesting. A small portion in the middle of the field of Tutsham plants had been planted with certain seedlings (*M.* 45, etc.) and the following year a big outbreak of Mosaic Disease occurred among the Tutshams surrounding the imported seedlings. The seedlings and the diseased plants were grubbed during the following winter (1922–23).

The position of those seedlings was the centre of the area which embraced nearly all the cases of Mosaic Disease found during the three years of observation (see Fig. 1, p. 231).

All the evidence collected shows that the disease was introduced to that garden by these seedling varieties which did not themselves show the disease (carriers).

As regards observations in the other gardens they, too, gave support to the view that the Mosaic Disease of hops is an infectious disease, as grafting experiments have since shown.

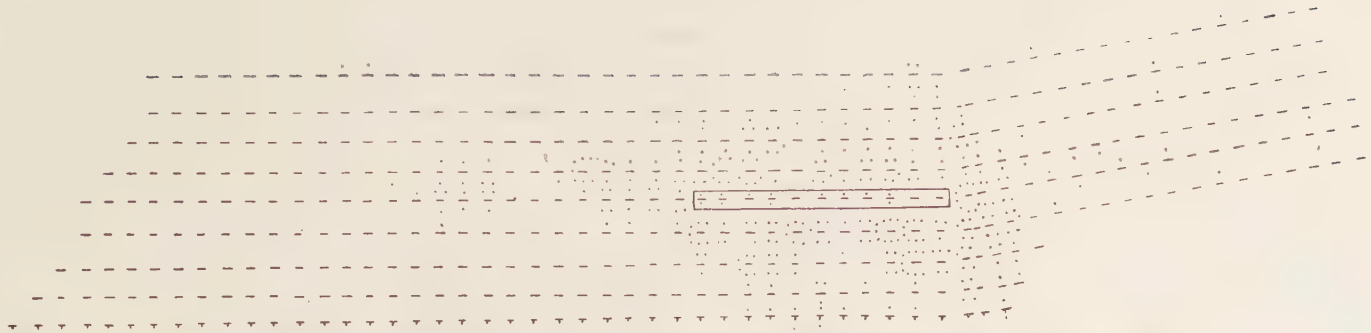

Fig. 1. Plan of garden *B* showing the position of hop plants developing Mosaic Disease during the years 1923–5. The diagram is approximately to scale. Each dot represents a single diseased plant. Each *short* line signifies 16 hop plants, and each *long* line 12 hop plants. The portion enclosed in the rectangle is that area in which certain seedlings, not Tutsham, were planted in 1921. One or more of these seedlings was a carrier of the disease. The other plants in this garden are all of the Tutsham variety.

Symptoms of the Disease.

The detection of the initial symptoms of Hop Mosaic presented considerable difficulty. Plants did not show symptoms until they attained the height of 3–4½ ft. Even diseased plants marked down and specially watched the following year did not exhibit symptoms until they reached that height. As a rule, the first indication of the disease was found in the

Fig. 2. Photograph of two shoots from the same plant (var. Tutsham). Left: the normal shoot. Right: deformed shoot from a diseased lateral.

leaves at the tip of the plant. A period of 5–7 days was quite sufficient for a plant hitherto normal to develop definite signs of bad Mosaic Disease.

Throughout the three years various types of mottling were encountered in all the gardens. They were classified and one special type is of importance. In 1923, two or three weeks before the picking, a big outbreak of this particular type appeared in all gardens under observation. The

mottling occurred, as a rule, on a few short laterals (usually no more than three) at the head of an otherwise perfectly normal plant. When these laterals bore hops, the cones were deformed and undersized (Fig. 2) and, in some cases, the mottled laterals died back at the tips.

Thirty-three cases of this mottling were found in one garden. The following year (1924) 21 were badly affected with Mosaic Disease; the remaining 12 were normal. In 1925 one of the 12 showed bad Mosaic Disease, while the 11 continued healthy in appearance.

In another garden, 30 cases of this same mottling were marked down for observation. In 1924, 20 developed definite Mosaic and were grubbed, 9 were normal, and 1 was suspect. The following year all 10 were normal.

The same type of mottling was found in 1924, and 10 cases found in one garden were watched. The following year 6 of them developed definite Mosaic Disease; the remaining 4 were normal.

These figures indicate that two-thirds of the plants showing this symptom one year will have Mosaic Disease the following year. The remaining third may be quite normal for the second and third years, or in a very few instances show definite Mosaic symptoms in the third year. The longest time any of these "mottled" plants may remain normal in appearance in succeeding years, whether any ultimately recover or whether they retain the latent form of the disease, has not yet been determined.

It should be stated that a large number of bad cases of Mosaic occurring in 1924 were perfectly healthy in 1923.

There is no doubt that this disease can be present in an apparently healthy plant of a susceptible variety, as the history of the following case shows.

1923. A one-year old plant.

June 8.	Regarded as a suspect.
July 6–8.	Definite *Mosaic Disease.*
Aug. 2–5.	Improved considerably.
Aug. 30–Sept. 4–5.	*Quite normal.* No mottling on the laterals and the cones perfect.
1924.	
May 5–8.	Normal (in any case too small for symptoms to appear).
May 15–18.	Normal.
June 11–12.	Suspect.

1924.	
July 25.	*Definite Mosaic Disease.* It had a "grow away" tendency, but the disease had the upper hand. It bore a small crop of deformed cones associated with definite Mosaic mottling.
1925.	Definite Mosaic Disease.

During conditions favourable for rapid growth diseased plants may produce growth free from disease symptoms, but they will not maintain the improvement after the conditions for rapid growth have passed[1].

Effect of Grubbing on the Control of the Disease.

With our present knowledge of the symptoms of the disease, prompt grubbing carried out over a period of two years will not entirely stamp out the disease, but it will check the spread and, in some cases, reduce the amount of disease in the third year.

In the garden to which reference has already been made in regard to the introduction of the disease by certain special seedlings, grubbing appears to have done much good.

The figures are:

1923. 104 cases: grubbed, 74; the remaining 30 were some of those showing the special type of symptom referred to on p. 232.

1924. 260 cases (240 new): grubbed, 259 including 20 of the 30 left in from last year. One left by error.

1925. 18 cases (17 new): these included the one left in from 1924.

That the low figures for 1925 may be due to the season masking the disease does not seem likely, since a garden about 10 miles away was very badly attacked with Mosaic Disease that year. Furthermore, an inspection in 1926 showed that the improvement was maintained.

In another garden of half the area where prompt grubbing was not carried out the figures for the three years are:

1923. 37 cases: grubbed, 4; 33 left in (all those showing the new symptom).

1924. 222 cases (201 fresh): grubbed, 164, about six months after the crop was harvested.

[1] This may account for a belief that a dressing of nitrate of soda will cure the disease. No cases were observed to recover permanently, and no correlation between manuring and incidence of the disease could be established.

1925. 83 cases (about 30 new): these included about 50 of those left in from 1924.

Unfortunately a strict comparison of this small garden with garden *B* is not allowable, since in the small garden there is the possibility that part of the garden had been planted with stock infected with a latent form of the disease, and further, a badly infected garden adjoined it, although that garden was being rogued of the disease.

Recommendations.

The disease is infectious in the field and for that reason prompt grubbing is to be recommended.

Prompt grubbing carried out for a period of two years will not stamp out the disease but will hold it in check.

Where a grower decides to rogue his garden of diseased plants he should examine the plants at least twice in the season; firstly when the plants are about 4–6 ft. high (*i.e.* at the breast wire in the Butcher System of training), and a second time two or three weeks before the picking.

Growers should be extremely careful to avoid the importation of "carrier" plants into their gardens.

Growers should avoid taking sets from infected gardens, also in infected gardens the practice of filling up gaps with cuttings from neighbouring hills cannot be too strongly condemned, as the disease may be present in a plant which is perfectly normal in appearance.

(*Received August 19th*, 1928.)

OBSERVATIONS OF THE *HELMINTHOSPORIUM* DISEASES OF CEREALS IN BRITAIN

I. THE BEHAVIOUR OF *HELMINTHOSPORIUM GRAMINEUM* IN A COMMON BARLEY DISEASE

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(With 3 Text-figures.)

CONTENTS.

1. Introduction.

This paper is an account of part of a re-survey of the *Helminthosporium* diseases of British cereals. The work was commenced in 1922, at the suggestion of Mr F. T. Brooks, and was mainly carried out at the Botany

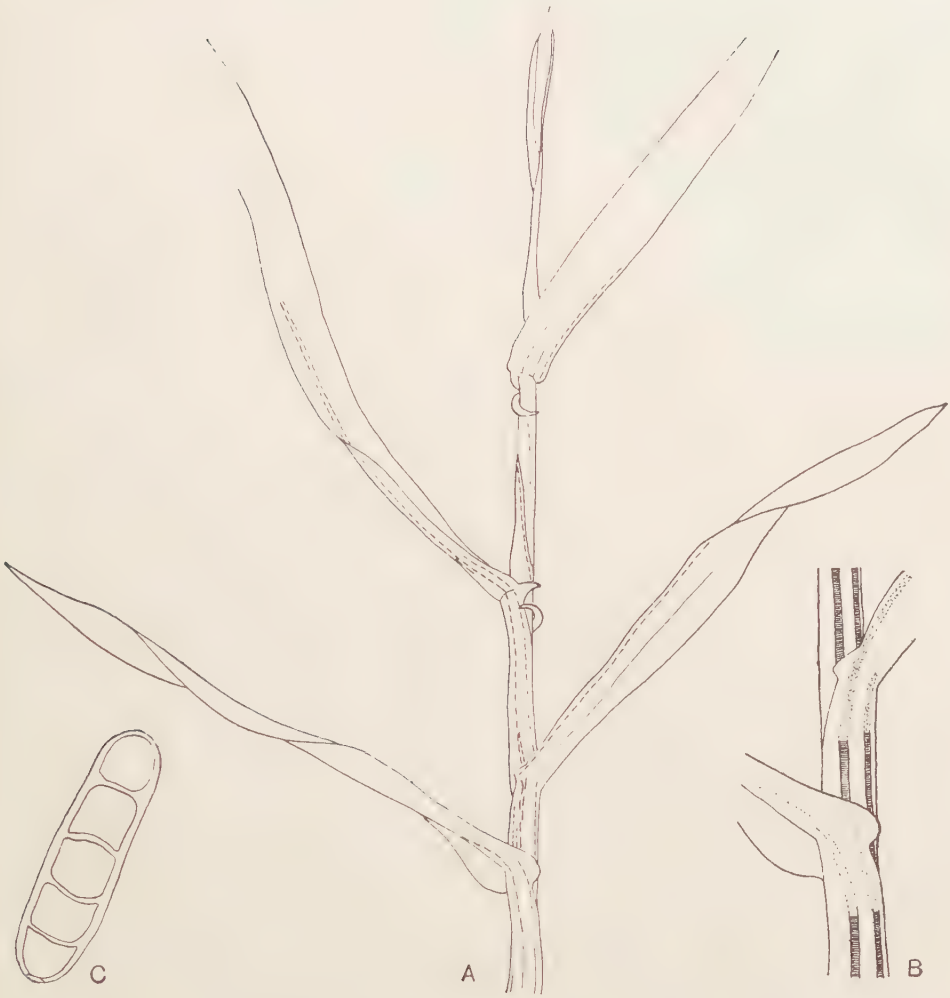

Fig. 1. (Drawn from a typical specimen.) (*A*) shows the upper part of a barley plant. The dotted lines represent the stripes of diseased tissue. Attention is particularly called to the tightly-wrapped sheaths. For part of the plant these are shown on a larger scale in (*B*).

In (*B*) the stripes, on the tightly-wrapped portions of the sheaths, are represented by heavy shading. Note how near the heavily-shaded portions of the second leaf are to being in line with the similarly shaded parts belonging to the first leaf.

(*C*) is a typical conidium of *H. gramineum*.

School, Cambridge. For Mr Brooks' constant helpfulness during the investigation I have here to record my thanks. The disease which is particularly dealt with in this paper is commonly known as Leaf-stripe Disease of barley. Fig. 1 will recall the characteristic form of damage, and the equally characteristic appearance of the commonest spore-form of the fungus.

The most convenient starting-point is formed by the work of F. Kolpin Ravn (7, 8). His work at once brought together the scattered and highly contradictory evidence, which had accumulated prior to 1900, concerning the *Helminthosporium* diseases of cereals, and compacted this by new discoveries. When the existence of two separate diseases of barley and one of oats caused by fungi of this genus had been made clear by Ravn's papers, the work of many authors made it apparent that the distribution of the diseases was very wide, and that their economic importance made them worth combating. Bibliographical references to these records of the distribution of the diseases, and of control measures which have been successful against them, are not given, the information being available elsewhere, notably in Drechsler's (1) paper.

When the conclusions of one worker, in this case Ravn, who of necessity dealt only with a section of one country, are utilised as a foundation for world-wide records, that foundation, naturally, is very severely tested. Conditions are rendered worse when the additions are made indiscriminately, resting completely on parts of the foundation which Ravn himself did not guarantee, or on some part which has been subjected to an ill-judged modification, or on some part which was guaranteed but was in reality inaccurate. In short, the mass of data concerning these diseases which has accumulated during this century is quite as formidable, in its confusion and insecurity, as the mass which confronted Ravn at the close of last century.

The two different diseases of barley which Ravn distinguished are (1) Leaf-stripe Disease, caused by *H. gramineum* Rabh., and (2) Net Blotch Disease, caused by *H. teres* Sacc. Among the important characteristics of Leaf-stripe Disease, which are not shown by Net Blotch Disease, Ravn pointed out that when the disease appears on one leaf of a plant then all the later leaves will show the disease, the stem and ear also becoming affected. Ravn also pointed out that infection of the seed is the only mode of initiating Leaf-stripe Disease in a plant.

As regards the host-parasite relation which is reflected in the symptoms, and in the mode of spread of the diseases, he concluded that *H. gramineum*, like certain of the Smut fungi, inhabits the growing-point of the

host plant, and thence spreads to each young part of the plant, during the formation of the part. Drawing a contrast he pointed out that *H. teres* mainly spreads, like the Rusts (Uredineae), by wind-borne spores.

Certain adjustments of Ravn's point of view have been suggested in recent papers, *e.g.* by Vogt (13) and by the present writer (10). References to these and others will be made in the appropriate later paragraphs. Several papers which are of considerable indirect interest, as they deal with the validity of various characters for distinguishing *H. gramineum* from other species, are not mentioned here at all, inasmuch as the British *Helminthosporium* diseases of cereals must be compared with one another from several points of view in a later paper.

In the present paper the host-parasite relationship in Leaf-stripe Disease alone is dealt with. This is the first full presentation of facts which show that the parasitism of *H. gramineum* is fundamentally different in type from what has previously been supposed. It does not lead a Smut-like life, but a life which is interesting in its own way.

It has already been noted that Ravn, following up an earlier suggestion of Rostrup (9), came to the conclusion that the mycelium of *H. gramineum* inhabits the growing-point of infected plants, and from there spreads to all the young tissues as they are formed. Of all his conclusions this would seem to be the one most requiring re-investigation. For even a casual glance at diseased plants raises doubts. If the mycelium causes this sickening and death of cells, wherever it penetrates in the leaves, sheaths, etc., will it not then also kill invaded growing-point tissues? The appearance of plants dead or dying from the disease certainly suggests that it sometimes does. Or, why does the fungus spread in the leaves only in stripes, and stripes which, as will be seen later, are rather peculiarly arranged, if all parts have an equal chance of infection? These are only two of the most obvious doubts which present themselves. One also felt that the Smut analogy had led Ravn into an inadequate perception of the seed-borne sources of the disease, but these sources are discussed fully in some later paragraphs.

2. Methods of Investigation.

Though field observations yield valuable evidence as to the host-parasite relationship, the main basis for its understanding must be supplied by microtome sections. No complex *résumé* of methods experimented with is necessary. It must suffice to say that satisfactory sections were obtained when ordinary fixatives (*e.g.* Chrom-Acetic) were used, and ordinary stains (*e.g.* Heidenhain's Haematoxylin, with counter

stains such as Congo Red). Many of the main facts can also be shown up by the use of Lacto-phenol and Cotton blue.

Cases of the three *Helminthosporium* diseases which affect barley in Britain, viz. *H. gramineum*, *H. teres* and *H. sativum*, were under observation during the present investigation. Parallel studies of the three organisms in pure culture and in infection experiments were also carried out. This gives a great measure of security against confusion regarding the identity of species.

As it is much more easy to differentiate *H. teres* and *H. gramineum* by their mode of growth in culture than by the symptoms which they cause in affected plants, it was essential to establish the main facts from plants grown from clean seed and artificially inoculated with the respective fungi. When naturally-infected plants had to be used as a source of material the identity of the *Helminthosporium* and its connection with the hyphae observed in sections was made certain. When the surface of infected tissues is sterilised, the internal mycelium readily grows out into culture media. After considerable knowledge of the conidia, as produced under various conditions, had been gained they also became a useful means of identification.

3. The Sources of the Attack on the Germinating Grain.

The sources of attack carried in or on the grain are (1) conidia, (2) mycelium, and (3) sclerotia, which may develop into perithecia.

(1) *Conidia* (or conidiophores) may be present on the grain whether it was produced on a diseased or on a healthy parent. This source was considered by Ravn(8) to be mainly responsible for Leaf-stripe Disease. Other sources will be considered later, but these, though more effective in the production of diseased plants, are less widespread. Conidia are produced so abundantly that even a few diseased plants might well contaminate a great proportion of a crop, during harvesting, stackbuilding or threshing operations. There is fortunately, however, little certainty of infection from this source. Ravn obtained ten positive results from 200–250 inoculated grains. The chance of infection from natural contamination with isolated spores must be considerably less than this.

In the closely adherent chaffs of the barley there is normally only one gap through which the fungal germ-tubes may readily enter, or through which the young barley shoot may push its way on germination. This is the aperture left by the incomplete overlapping of the pale by the flowering-glume, at the apex of the grain where the base of the awn is.

In this vicinity conidia may obtain lodgment, and, putting out germ-tubes when the seed is sown, may reach the coleoptile of the emerging shoot. Elsewhere the chaffs give less opportunity for the lodgment of spores and form a barrier which must be circumvented by the germ-tubes. Zade (14) similarly dismisses as slight the chance of effective penetration by spores of *Ustilago avenae* present on the outside of oat grains.

(2) *Mycelium in the Chaffs and Pericarp.* When the fungus is considered in relation to the flowering stage of the barley it will be shown that this awn-end of the chaffs is the part which is most likely to contain mycelium as well as conidia. This is because the presence of such mycelium in the grain-coats results either (1) from the ear of a diseased plant brushing against the fungus-containing sheath (of the last leaf) during its upward passage; or (2) from a grain of a healthy plant being infected, while the chaffs were still soft, by a spore from a neighbouring diseased plant.

That mycelium could be present in the chaffs of germinable grains from the first source did not appeal to Ravn as being other than a rare possibility. He believed that plants with Leaf-stripe, as with Smut, rarely produce germinable grains. From the attitude which is taken up in the present paper it will be apparent, especially later, that the grains which are produced on diseased plants are to be considered as liable to show all degrees of infection, those showing slight, moderate, or even fairly severe infection being germinable. Thus the importance of mycelium from the first source has now to be considered greater.

That mycelium could be present in the chaffs and kernel of germinable grains from the *second* source was mentioned by Ravn (7). Vogt (13) further emphasised the importance of this mycelium, but it is by no means certain that he was dealing with *H. gramineum.* Ravn did not describe the nature and distribution of this mycelium. If he investigated it at all, his attitude towards it must have been very different from mine. Observations of the mycelium in the chaffs in some cases, in the kernel in others, and in the embryo in still other cases, he must simply have considered as observations of the progress of the fungus nearer and nearer to its proper goal, inasmuch as he considered that the infection of the embryo is the first step in the initiation of Leaf-stripe disease. I consider, on the other hand, as will shortly be seen, that an embryo which has mycelium in its near neighbourhood is far from likely to produce a typical Leaf-stripe plant, and is, in fact, likely to produce no plant at all.

From either source, then, the quantity of mycelium which is able to establish itself in the chaffs, and in the inner parts of the grain, is liable to vary greatly. A very small quantity, causing only very slight discoloration of the chaff, is sufficient as a source of the disease. At the other extreme, when the endosperm, and even the embryo, is invaded, the quantity is so much more than sufficient that the plant is overwhelmed either before germination or very shortly after. While the lightly infected grains must be the source of a far greater number of Leaf-striped plants, yet these cases which are but one stage better than ungerminable seed are also illuminating as to the nature of the fungus.

The region in which the *Helminthosporium* hyphae find a passage from the outer seed-layers to the endosperm is at the basal end of the furrow of the grain where the end of the aleurone layer abuts on the scutellum. Elsewhere the crushed remains of the seedcoat, cross-cells and tube-cells usually form an effective barrier to the penetration of the aleurone layer. Its slow progress in closely packed tissues is a characteristic of *Helminthosporium*, exemplified in the behaviour of mycelium penetrating the embryo through the scutellum. Closely packed cells have immediately to be met, and the fungus from this source is often left impotent for a considerable time, only assisting in the general lowering of the vitality of the plant. This is more effectively produced by the fungus from other sources, which have now to be considered.

Mycelium in the chaffs and pericarp, we have seen, is inevitably present when the endosperm is invaded, and may be present even when the endosperm contains no fungus. This mycelium in the outer seed layers can remain viable for at least two years and under the damp conditions of germination produces conidia and fresh hyphae. These are the effective agents in penetrating the primary sheaths, *i.e.* the coleorhiza (root-sheath) and the coleoptile (plumule sheath). Penetration of the coleorhiza, or of the rootlets which arise from it, results in rotting, stunting, or, at the least, in impaired efficiency of the root system, which is a contributory cause of sickliness in the plant.

Penetration of the coleoptile, on the other hand, is the first step towards the production of Leaf-stripe. The first opportunity for such penetration occurs when the shoot, covered by the coleoptile, commences to push its way between the inner surface of the chaff and the outer surface of the kernel. On one or both of these surfaces fresh fungal hyphae may be developing. The young shoot is further menaced at its point of emergence from the chaffs, since here may be met hyphae spreading on the outer surface of the chaffs.

The form in which this important chaff mycelium overwinters is interesting. Much of the mycelium becomes changed into a very resistant form, with thick walls and the cells short, often swollen. This resistant mycelium would deserve more detailed consideration were it not that Vogt (13) has described, at considerable length, a similar mycelium in chaffs affected by the species of *Helminthosporium* with which he dealt. The same type of resting mycelium occurs in cultures when the medium is drying up, and is also known in other fungi (particularly those tending to produce sclerotia) when the environment is becoming unfavourable for further vegetative growth.

(3) *Sclerotia and Perithecia.* It is probable that all the spherical or pyriform sclerotia which are formed on the surface of grain tissues, or within them, can be regarded as potential perithecia. On the other hand, it is doubtful whether ascospores are formed often enough, or early enough, to be a considerable source of danger to the young plants. In this very resistant form, however, the fungus is likely to be more difficult to kill than the embryo itself, *e.g.* by seed-disinfectants, and it is certainly capable of producing numerous conidia and fresh hyphae under the moist conditions of germination. This "resting-stage" is thus of practical importance.

In a paper published during the course of this work, van Poeteren (5) describes the production of sclerotia of "*Pleospora trichostoma*" on the outside of diseased grains (from Leaf-stripe plants) when these have been kept for three days in a damp atmosphere. The only sclerotia which I have found definitely associated with the *Helminthosporium*, under these conditions, have been of the rather unorganised type which Noack (4) terms "nests of mycelium." There are undoubtedly sclerotia present in or on some diseased grains throughout the winter. Early stages of ascus-formation which were seen in some of these will be described later.

4. The Possibility of Seedling-infection from the Soil.

It is necessary to consider the soil as a possible source of fresh outbreaks, but that consideration can be brief. The fungus undoubtedly reaches the soil in as many forms as it reaches the chaffs. There are several viable forms present in ground which has been recently cleared of a diseased crop, and the viability of the sclerotia at least persists for some considerable time. Yet these viable forms, by the ploughing activities of the farmer and the competing activities of micro-organisms will be placed for the most part in a position unfavourable for attack on a new crop. Even if the fungus in some viable form were favourably placed, *e.g.* very

near a germinating seed, still it would not have more chance of initiating Leaf-stripe disease than conidia on the outer parts of the grain have. The chance of infection from these conidia has already been dismissed as slight.

It is an undoubted fact, as previous authors have mentioned, that the distribution of Leaf-stripe plants over a crop is sporadic, not in marked patches. This suggests, among other things, that Leaf-stripe is not a soil-borne disease. One agrees with Drechsler (1) that Johnson (3), who greatly emphasised the capacity of *H. gramineum* to infect from the soil, was mistaken in his identification.

5. The Fungus in the Sheaths and Leaves of the Seedling.

Penetration. From one or other of these sources, then, mycelium comes into contact with the coleoptile. When the mycelium has grown to some length, passing along the outer surface of this sheath, appressoria are formed, and a narrow "penetration hypha" pierces the host epidermis. The subsequent course of the penetrating hypha is, in my experience, between epidermal cells. Noack (4), Ravn (7) and Stevens (11) record similar observations with kindred species of fungi and host-plants. Some observations show that the first attacked cell is invaded by mycelium, but thereafter the progress of the hyphae is fundamentally intercellular; the cells of the host are not invaded till they are in a very unhealthy condition. Such confirmation of Ravn's conclusion that the progress of this fungus is fundamentally intercellular was suggested as necessary by Stevens (12), when he described the very different behaviour of *H. sativum*, an *intra*cellular parasite.

The Fungus in the Coleoptile. The coleoptile is mainly composed of thin-walled cells, vascular bundles being present to the number of only two. The proportion of tissue easily traversed by the fungus is thus greater than in a leaf, and the result is more speedy invasion and a diseased area not strictly delimited as a stripe. Owing to the arrangement of tissues, however, the progress of hyphae in the cell walls which are parallel to the long axis of the shoot is uninterrupted, while progress towards the inner surface of the coleoptile must be effected by a zig-zag course. As would be expected from this, the fungus makes more progress upwards and downwards, from the point of penetration, than radially. The time elapsing before the inner surface of the coleoptile, which is in contact with the outer surface of the first leaf, is reached will vary according to whether conditions are in favour of host or parasite, *e.g.* in the number of hyphae which have effectively gained entry.

In general it is found that hyphae have reached the epidermis of the coleoptile, have broken through it, and are present on the moist inner surface, while the first leaf is still growing up in contact with this surface. Penetration of the leaf (or its sheath part) thus occurs on its outer surface either before or soon after its emergence, just as did penetration of the coleoptile from the adjacent chaff or grain tissues.

The other possible mode of attack, where the hyphae invade the leaf from the node which produces it, must depend on the possibility of the mycelium travelling down the coleoptile to its base, then up the stem internode to the node of the first leaf. The amount and nature of the tissues that would have to be traversed either renders the threat from this source abortive or results in hyphae gradually making progress in the meristematic regions. As in the case of mycelium penetrating these regions from the scutellum, these hyphae cannot be regarded as effective in producing Leaf-stripe in new leaves, but can be regarded as contributing in a greater or less degree to sickness or death of the plant.

When the first leaf passes up through the fungus-containing coleoptile the possible results for the leaf may be summarised as (1) death (meristem invaded), (2) Leaf-stripe (lateral invasion), (3) escape. The second leaf wrapped up within the first has in turn to face the same possibilities, and so on for all leaves, sheaths, and finally the young ear. Death may come with varying speed, Leaf-stripe may be developed to a varying degree, and escape may be temporary or permanent.

6. The External Manifestations of the Disease and their Interpretation (Earlier Stages).

A general idea of the potentialities of the fungus, working within the host, has been gained by considering in detail the processes which result in the infection of the earliest invaded parts. As the plant grows older the results of these activities become accessible for macroscopic examination, and this large-scale study yields evidence corroborating that of the microtome sections.

Changes in the Leaf. Typically the young leaf on emerging, or soon after, shows the presence of the fungus in one or more stripes. These are, at first, only apparent locally, and distinguishable mainly because of their translucency as compared with the neighbouring healthy tissue. This was noted by Ravn (8), though perhaps with too definite an emphasis on the earliness of the appearance of this stage. The time required for a sufficient massing of the fungus to produce this macroscopically evident system, indeed, varies greatly. After this, and before the often-described

stage of the brown "mummified" stripe is reached, there is a definite stage when the stripes are distinguishable only by their loss of green colour. According to Ravn(7) the internal disorganisation, which is associated with this loss of colour, is confined to the mesophyll. "The chloroplasts lose their colour to a greater or less extent, and may be altogether destroyed.... Two types of hypha are present in the mesophyll, (1) hyphae which are quite straight for long distances, and (2) branches from these long hyphae, which, with a more or less curved course, distribute themselves, mainly in the transverse direction, throughout the mesophyll." The full significance of the abundance of the long hyphae is considered later.

It is worth while to emphasise further a point which has already been implied in the summary of the microscopic evidence, which was given at the end of the last section. The point is that even in a leaf which is showing only the "pale stripe" stage, the fungus is in a position to penetrate a new leaf.

From the quotation which has just been given, it does not seem that Ravn would have considered such a contention unreasonable. Not having looked at the distribution of the mycelium from this point of view, he limits himself too much to a description of the mesophyll damage. From the main mass of hyphae in the mesophyll a few, at least, of the transverse branches do pass between the cells of the inner epidermis, and so come into contact with the surface of the next leaf. The fact that, when a "pale-striped" leaf is kept in a damp atmosphere, mycelium grows out from the stripe into the air, often in quantity, may also be commented upon.

The mere starvation of the affected tissues, which must result from the presence of so many fungal hyphae, could probably account for this loss of colour, just as starvation, in the absence of any pathogen, can cause yellowing of leaves in general[1]. Though Ravn uses the rather more non-committal terms "afblegningstadium" (Dan.) or "Verblassungsstadium," yet one feels that the term "yellowing," with reference to this phase, is not inapplicable. It has been my experience that the "yellow-stripe" stage, which, quite often, persists as the only sign of the disease on plants showing several leaves, has proved puzzling to many observers. This is not surprising, in view of the scant notice which it has received in most published accounts of the disease.

The time of appearance of the first brown areas, within the pale stripe,

[1] I am indebted to Dr H. Godwin for suggesting this as a corollary on his own work on the physiological aspects of the yellowing of leaves.

thus varies and the spread of the "mummification" is also somewhat erratic. Typically, however, a diseased leaf shows, within a few weeks of its unfolding, the brown strip extending for practically the whole length of the leaf. A clear idea of the final state of the stripe—brown, shrivelled and bearing conidia—is given by Ravn's account.

7. THE STRIPE FORM.

An investigation of the reasons for the disease appearing in this stripe form yields interesting results, and comparison with some Smut-diseases, which also produce lesions of the stripe form, on leaf and sheath tissue, shows that the distribution of the stripes, in Leaf-stripe disease, is dependent on factors different from any which have been noted as operating in these other diseases.

One factor operating in this disease, in common with most other stripe-forming diseases of the Gramineae, is the *mechanical resistance* offered by the strengthening tissues, which are present in and around the vascular bundles. It is clear that these bands, arranged lengthwise in the leaf, form a bounding line which is only slowly overpassed by the mycelium. The vascular bundles of very young leaves form a less effective barrier, and also bundles which have been involved in the general disorganisation of tissues in a "mummified" stripe.

It has been seen that in the coleoptile the influence of such tissue is little felt, but that the arrangement of cells tends to promote longitudinal spreading of lesions. (Macroscopically this is not usually very clear, as the "mummification" is only contributory to the natural collapse of the sheath quite early in the life of the plant.) Thus, when hyphae break through the inner epidermis and come into contact with the first leaf, *penetrations are likely to occur on a longitudinal strip or strips*, corresponding to those on the coleoptile. The upward growth of the leaf will also *brush a vertical strip* against the externally applied mycelium. The inrolled part of the leaf will escape penetration at this time, as will a greater or less proportion of the exposed surface, owing to the variable amount of mycelium which will have traversed the coleoptile.

The *sheath part* of the first leaf will be infected, just as is the blade part. This sheath part in the first few leaves, the "basal rosette," is much less strengthened than in the later leaves, a point which is in favour of the fungus. Though the sheath-stripe may remain in the yellow stage for a much longer time than the blade-stripe, it is still at that stage capable of infecting the young second leaf which passes up inside it. Its more lasting contact with the coleoptile renders it more liable than the leaf

blade to infection, and its closer relation to the second leaf makes it more potent in infecting. The inevitability of the presence of this sheath-stripe has not been appreciated, the literature giving rather the impression that it is a phenomenon that may or may not occur.

8. The Correlation and Distribution of the Stripes.

The stripe on the second leaf would be expected to occur in the region of that leaf which was in contact with a stripe area on the first leaf. In the absence of complicating factors, some of which are noted below, this correlation is clearly seen, as in the plant figured (Fig. 1). It is more obvious in the cylindrical sheath parts of the successive leaves of an elongated shoot, since the arrangement of the still folded parts is nearer to that in a drawn-out telescope.

On the unfolding of the leaves, especially the later ones, it is commonly noticed that the stripe on the young leaf is broader than that on the preceding one. [In Ravn's (7 and 8) coloured plate of the three uppermost leaves of a diseased shoot there are indications of this.]

The mycelium from the older sheath has been able to spread, either (1) before infecting the young leaf, *i.e.* on the surface of contact between the two leaves, or (2) after invasion of the young leaf has occurred. Lateral spreading during the second period will only be considerable if the leaf is penetrated while it is still very young. This will be realised from what has already been mentioned concerning the confinement of spread, on the part of the newly established fungus, to the line of least resistance, and concerning the vascular barriers which early become effective.

With reference to the question of spread on the surface of contact between the leaves, it is of interest that Raunkiaer (6) found that the narrow space between the sheath and haulm (of grasses) was constantly moist. He believed that the ligule assisted in the maintenance of this condition. The presence of moisture on the inner surfaces of sheaths, the outer surfaces of which are exposed to aerial drying, will render circumstances more favourable for the emergence of hyphae on the inner side of the sheaths, and especially for the spread of hyphae on this surface. Experience gives one the impression that such spread is mainly responsible for the successively broader stripes in the leaves and sheaths, passing from the lower to the upper.

Among the cases where the width and number of stripes increases as successive leaves are unfolded, there are some in which diseased tissue is apparently absent in the first leaf or leaves, in the first and second for example, throughout their whole life, yet a narrow stripe appears in the

third leaf, and the disease gains an increasing hold on all the later leaves. These cases are of interest since they suggest, at first sight, that there is some justification for the claim, made *e.g.* by Drechsler (1), that this fungus, like the Smuts, can be present in the growing-point of the plant, even when no outward symptoms are visible. In all cases of this type which I have examined, however, mycelium has been found in the sheath portions of the earliest leaves. Mycelium in this situation, it has been noted, is dangerous though not conspicuous, and can be present without the necessity of mycelium being present in the blade. One must agree with Drechsler that in no case is the appearance of typical symptoms in the later leaves, of an apparently healthy plant, the result of secondary infections.

The observations recorded in the foregoing paragraphs all point to one conclusion, in addition to those already mentioned, viz. that the adult leaf is not a favourable environment for the spread of the fungus. Both as regards penetration from the outside (secondary infections) and as regards the spread of internally-present mycelium, the adult tissue is clearly much more resistant than the tissues in which the fungus first establishes itself.

One is rather puzzled by Ravn's observation that "little by little the mummification spreads over the whole leaf-surface, the green colour of which can only be observed as narrow stripes along the edge" (7). It is clear that, if this were constantly to occur with every leaf, one could hardly observe many of those cases which have been noted in recent paragraphs, *e.g.* the much narrower stripes on the much older leaves. It has rather been my experience that mummification, little by little, overspreads that portion of the leaf-surface which has shown the pale-stripe stage.

The only marked extension of the diseased tissue, in an adult leaf, takes place at the base of the blade. Extension there results either from the spread into the blade of a "late" sheath-stripe, or from the germination of conidia in the basin occurring at the base of the blade, where the auricles clasp the haulm. Yet, even from these sources, the progress of the *Helminthosporium* mycelium upwards, into the drier and tougher parts of the leaf, is slow.

9. Escape of the Later Leaves.

Plants which continue to produce a series of striped leaves (and, ultimately, a more or less diseased ear) have mainly been kept in view up to the present, since they provide the fullest demonstration of the working

of the fungus in the host. Departures from this type, however, frequently occur, and these, because of their interest, will have to be referred to from time to time.

Plants which are affected with this disease may cease from producing the normal series of striped leaves, from either of two causes. Firstly, death may intervene at any stage of the life-history of the host, from causes already described. Or secondly, the later leaves of the plant may escape.

Such cases of permanent escape are fairly frequent. For instance, in an experiment which involved a close watch on 113 plants, which showed infection of the lower leaves, 26 plants showed escape of the upper leaves and ear. In such cases the fungus had a bad start, and has ever since failed to "live up to the time-schedule" necessary for the production of a fully diseased plant. It will be realised that, on occasion, conditions may favour the overcoming of this initial lag. More often, however, the lag is accentuated.

10. The Fungus in the Stem and Growing-point.

When one looks, then, at the leaf symptoms of various plants, one obtains interesting evidence. When one examines the mature stems and the exposed ears of diseased plants also one can deduce with fair safety the sources from which the stem internodes and the ears become infected. This is true though the actual infection takes place before ever the internodes and ear become exposed to view.

Thus for giving the simplest picture of the course of the disease it would scarcely be necessary to discuss what happens while the stem and the young ear are still hidden within sheathing leaves, although one is compelled to discuss these happenings to some extent, for the sake of thoroughness, but, still more, because Ravn particularly chose, as his most striking evidence, cases at this stage of development.

11. Infection of the Stem.

Any leaf (*a*) arising at node (*A*) has the lowest part of its sheath in contact with the internode *AB*, and the remaining part in contact with the sheath part of the leaf which arises from the succeeding node (*B*). In a diseased plant the leaf arising at *A* will become infected from the one which ensheaths it, and in due course its surface which is in contact with the above-named parts of the stem and sheath will transmit the fungus to them. From this source, then, each stem internode when in a more or

less extended and hardened condition, is penetrated from the same source and at the same time as the leaf arising immediately above it. Mycelium from this source will not reach node *B* before it has reached the leaf (*b*) which arises from it, *i.e.* it will not be successful in initiating infection in the leaf through the (nodal) growing-point which gives rise to the leaf.

Internode after internode is thus infected from the "external application" of hyphae to it. After the "application" not unnaturally the hyphae spread in the stem, finding the epidermis and the pith in particular fairly easy tissues to which to travel. Mycelium thus reaches the pith of the successive internodes as a consequence of a vigorous mycelium being present in the leaves. It is one of the essential differences between Ravn's view and mine that Ravn considered that the mycelium reaches the leaves as a consequence of the presence of mycelium in the pith.

On any theory the first pith mycelium must be considered to have reached the pith from some form of "external application" (*e.g.* conidia germinating on the seed). Thereafter, on Ravn's view, the pith mycelium speeds on as the prime mover, leaving in its wake diseased leaves. On my view infection of leaves and stem-parts by "external application" continues, the diseased leaves leaving in their wake diseased internodes. Enough has been said to show how the facts concerning the distribution, for instance, of the stripes on the leaves fit my view. I cannot, in view of all the facts, regard the pith mycelium as more than a somewhat laggard co-operator which arrives to rot[1], to a small extent, the core of structures which are much more stoutly beset in their peripheral parts. In that case, one might almost say, there is being laid down a slow-spreading, tenuous, and by no means continuous stripe or stripes of diseased tissue. The stripe is confined to the core since the vascular bundles and some of the cortical tissue act as barriers. [It is significant that Ravn never found mycelium in the vascular bundles or cortex ((7), p. 27).] The slow spread and the by no means uninterrupted course of the stripe is the result of the closely packed tissues which have to be negotiated, particularly at the nodes. Even in very young nodal regions the hyphae are retarded.

The above mentioned conclusions as to the laggard nature of the pith mycelium are the result of much careful consideration of what was revealed by many microtome sections. To quote in more detail the data which these provided would be to make too much of the matter.

It would have been, perhaps, most satisfactory to compare the relative potency of the pith mycelium and the mycelium derived from

[1] Rot is perhaps too metaphorical an expression, but certainly, as Ravn pointed out, the walls of the cells surrounding the hyphae turn brown, the cells dying.

"external application," utilising the data which Ravn gives. Unfortunately he gives insufficient data. Of the two sets of mycelium whose

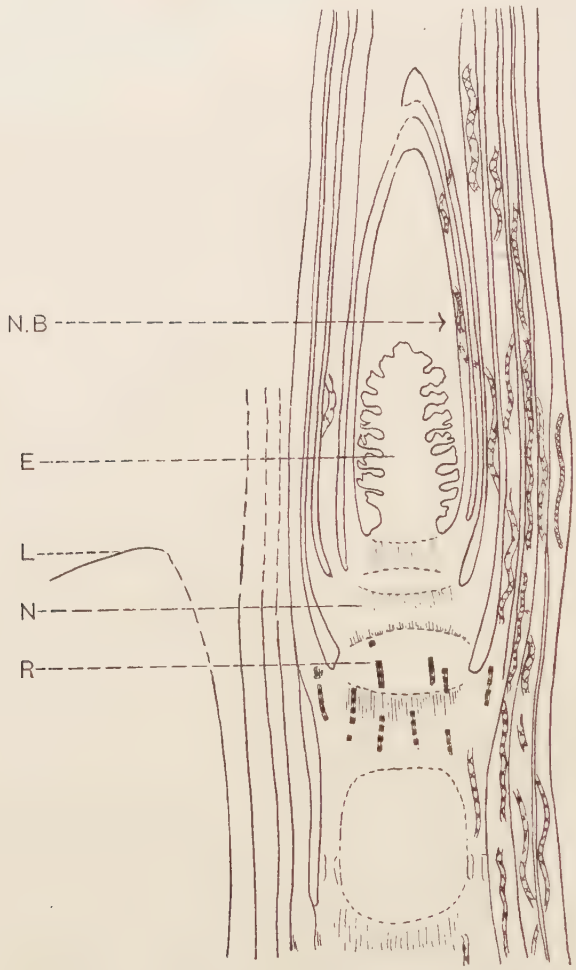

Fig. 2. Median longitudinal section of part of a young barley plant. The growing region of the stem is shown, enclosed in the sheath parts of leaves. The blade part of only one leaf (*L*) comes into the figure. The nodal regions of the stem are indicated by light shading, as at *N*. For further explanation see text.

position one would like to compare, he only shows in full the position of one, the pith mycelium. Therefore the comparison cannot be made.

This part of the work is illustrated in Fig. 2. The figure, built up from several sets of microtome sections represents a section of part of a barley

seedling. The four uppermost internodes of the stem are shown, together with the rudiments of the ear (*E*) which is forming at the stem apex. Such a young stem would, in nature, be hidden from view within a tube formed of the sheathing bases of leaves. The leaves involved would comprise both the leaves which arise from the four nodes included in the diagram and other leaves from lower nodes. These leaf-sheaths are indicated rather diagrammatically. The blade parts of one leaf (*L*) also come into the picture. The leaf parts which are infected by hyphae are traversed in the diagram by snaky lines, inserted for the most part only in the right-hand side of the figure. Mycelium within the stem is also present. The position of this is carefully marked in the region (near *R*) which is the uppermost limit of its spread. In that region it is represented by straight dotted lines.

A figure which Ravn gives, both in (7) and (8), shows that when he found a quantity of mycelium within the stem, exactly such as is indicated at the level of the arrow (*R*) he was convinced that he had found the mycelium which, after the fashion of Smut mycelia, infects all the young parts. How, in actual fact, the leaf mycelium is in a position to anticipate it in the infection of new parts is shown particularly in the region of the arrow (*NB*). The young ear is going to receive an "external application" of hyphae, from the uppermost leaf, long before the mycelium in the interior of the stem has worked its way up the rachis and commenced to damage the ear from its core.

It will be realised that, when the hyphae in all parts of the plant have made rather more progress than in the case illustrated, weighing of the relative importance of the two sources of mycelium, central and peripheral, becomes difficult. The difficulty is the same as that which one encounters when one examines, with the naked eye, a barley plant every leaf, every stem part and every grain of which gives evidence of the presence of the fungus. One cannot say without knowledge of the history of the plant whether the stem was infected from the diseased leaves which ensheath it, or whether the leaves were infected from the diseased stem which produced them. These wholly diseased plants, like Ravn's figure, represent final phases, which do not necessarily bear clear evidence as to their previous history.

12. The Consequences of the Infection of the Growing-point.

It has been shown, then, that first the leaves are infected, that from them the stem parts are infected in ascending order, and that, from both leaves and stem, hyphae converge on the growing-point. In other

words the whole order in which parts are infected is against the Smut analogy.

It has been stated also that the consequences of the infection of meristematic tissues is also against the Smut analogy, inasmuch as it has been stated that death (coming quickly or slowly) is the fate of leaves or whole seedlings when the meristem, which should undistractedly continue tissue formation, is laid under toll by fungal hyphae. It was stated that this sequence of events in the case of leaves or seedlings was deduced from microtome sections. It is of further advantage that this evidence should be confirmed when the activities of the fungus and their effects on the host tissues are alike demonstrable on a larger scale.

In viewing the large-scale activities of the fungus in the leaf the reader must have realised more and more that this is not a fungus which is likely to leave unharmed any tender tissues among which it may be dwelling. Even more pertinent evidence is provided, however, when the last internode, elongating at the time of the "earing" of the barley, becomes visible, to the naked eye. That this last internode, which ought to be long and straight, is in a diseased plant more or less stunted, and often so weak as to be cast into corkscrew coils, is well-known. There is good evidence to show that this distortion is the result of the fungus being present in the tissues of these internodes, the bulk of the damage having been done when they were young and tender. Some good evidence is afforded by statements made by Ravn. In the month of May he found that the position which the hyphae occupy in relation to the uppermost internodes was that represented by the position (*R*) in my figure (Fig. 2). In rather older stem-apices he found that mycelium had progressed as far as the uppermost internode. At the end of June he found that a great stunting of growth began. It is rather surprising that, in the light of these happenings, he did not interpret the fungus as being more of a disturber, and less like a symbiont.

13. The Fungus and the Young Grain.

The kind of menace which threatens a young ear has been described and shown (in the case of a very young ear) in Fig. 2. The number of the menacing hyphae, of course, varies, as does the degree of their success. Accordingly, for the ear, as for other parts, the three possible fates are (1) escape, (2) death, or (3) the reception of a greater or lesser quantity of mycelium. Ears meeting the third fate are the ones which interest us here.

When the state of the mature grains of such ears was being discussed

(in connection with the forms in which the fungus is present on the seed as sown) it was stated that the headquarters of the mycelium in the chaffs is at the awn-end. The reason for this was also briefly indicated, namely, that the mycelium had been "externally applied" by the sheathing base of the uppermost leaf. It is often clear macroscopically that the diseased areas on the chaffs of the newly-emerged ear are precisely those upper and dorsal parts which were in contact with the diseased parts of the enwrapping sheath, *not* the embryonic ends which are tucked in towards the rachis. The group of grains which Ravn figured ((8), Taf. 1) as being typical of the early stages of infection show this fairly well. When the ears emerge the lesions on the grains may be broad and long-established or small and newly established, the reasons for this variation being exactly the same as the reasons for variation in the leaf stripes. This variation in the degree of chaff discoloration is important for the man who has to select barley to plant as seed.

14. "BLINDNESS" IN LEAF-STRIPED BARLEY.

The ears of Leaf-stripe plants are commonly of the type known to the farmer as "blind" ears. In other words, they do not completely emerge from the sheathing base of the uppermost leaf. Nothing more than a summary of the reasons for this is required.

(1) The uppermost parts of the stem are weak. This weakness is contributed to both by the "externally applied" mycelium and by the mycelium which rots the core. In connection with the former it may be noted that the uppermost parts of the stem are commonly the most completely invaded, just as the uppermost leaves are. The uppermost internodes, because of this weakness, cannot play their part in thrusting the ear out from the confining sheath.

(2) The forces which retard the emergence of the ear from the sheath are also, in diseased plants, abnormally great, since the auricles of the uppermost leaf are hard and mummified, forming a barrier across the natural exit of the ear.

These two causes combine to produce the imperfect emergence of the ear. Ravn considered that such imperfect emergence is peculiar to Leaf-stripe disease (*H. gramineum*). A similar imprisonment of the ear may, however, result when *H. teres* causes "mummification" of the auricles of the uppermost leaf. Outside the genus, various fungi which affect the auricles in this way, and cause also a greater or less weakness of the straw, may produce this symptom.

15. The Disease and the Tillers.

The tillers which arise from a diseased main shoot may escape, may be killed, or may live infected to a varying extent. The re-iteration of these three possible fates may seem platitudinous but, in point of fact, one gathers from the existing accounts that the third of these fates is inevitably met. On general grounds alone the reader of the foregoing paragraphs will immediately question this. Our fungus is not one which inevitably allows to live any organ which it invades, nor is it one which inevitably succeeds in infecting an organ, even though the older organs which enwrap or ensheath that organ contain a well-established mycelium.

Speaking of the case of the tillers in more particular terms, one may note the following facts. The source of infection for the tillers which are produced on a diseased main shoot, is the mycelium contained in the sheath part of the leaf which subtends the tiller. (To a lesser extent, of course, any other diseased part with which the tiller may come into contact can contribute.) As to why mycelium from that source need not always be successful, various reasons might be advanced. The fungus may not have moved in the leaf to a position favourable for invasion of the new part. Or, again, the fungus, by reason of the competition of other micro-organisms, or the accumulation of its own waste products, may be in no condition to make new progress.

16. The Tillers which Escape.

The possibility of escape is most often realised in the case of late tillers. It is more important and hopeful, from the practical point of view, to note that tillers frequently escape early enough in the season for them to produce useful ears, so that in the field it is something of a shock to find, at the base of fine healthy shoots, the ruins of others killed by Leaf-stripe.

As to how often, in actual field observations, escape of tillers can be found, my experience differs from that of Ravn. He records(7) that of 396 diseased plants he found only 3 per cent. with any sound tillers. The percentages which I found were higher, *e.g.* in one series of observations, dealing with 215 diseased plants, I found that 42 per cent. had at least *some* tillers sound. Such discrepancies between his work and mine may partly be explained by the difference in the conditions of the experiments, *e.g.* as regards climate and type of seed.

17. The Time for Observing the Incidence in a Crop.

Probably, however, the main reason for the discrepancy is to be found in the fact that Ravn's count was made at one period of the season only, viz. soon after earing ((7), p. 114). In such a count, the remains of tillers which have been killed by the disease, are very easily missed at the base of fine healthy tillers. Since this point concerning the time of counting is important from the point of view of getting correct results in infection experiments, for instance, or susceptibility trials, it may be added here that a count too early in the season is still more unreliable. Even for the purpose of classifying plants simply as infected or uninfected, it is not advisable to make a count before the barley plants have reached the "rosette stage." At earlier stages the disease, if not actually lagging out of sight, is present in atypical or inconspicuous forms. If the most accurate results are desired, a "rosette-stage" count should be supplemented by one taken about the time of earing.

18. Infection Experiments.

From considerations of space, several questions concerned with artificial infection can be left over to a later paper, in which they can be discussed as being common to several species of the genus. Among these questions are the devising of efficient means of infecting the germinating grain, and also the whole topic of "secondary infections." Secondary infections of the leaf are not an important source of damage, so far as this disease is concerned, while secondary infections of the young grain have not been quite fully investigated.

Disease-intensity under Varying External Conditions.

Certain results, however, concerning the influence of conditions on the disease, results derived both from infection experiments and from general observations, are intimately connected with the main theme of this paper.

Soil-temperature at germination. Many observations of mine confirm what is already known from the results of Ravn (7, 8) and Johnson (3*a*), namely that when seed which harbours the fungus is subjected to low temperatures at the time of germination, the chances of a diseased plant being produced are greatly increased.

Low temperatures greatly slow down the germination, increasing the chance of the fungus effecting an entry while the young shoot is still confined within the chaffs. The development of all parts of the

plant may be similarly retarded by low temperatures during the later life of the plant. Thus temperature undoubtedly has a bearing on the question of how many plants will, completely or partially, escape. It may be noted that, according to Ravn, the fungus makes slow progress even at 3–5° C.

A New Attitude Concerning the General Effect of Conditions.

It has already been emphasised that a great difference between the course of the disease, as here described, and the Smut-like course which was ascribed to it by Ravn, lies in the fact that a plant, which has been infected in its early stages, must now be considered to be able, by vigorous growth, to grow away from the fungus. Sowing when the soil-temperature is relatively high (on British standards) is one way of promoting this vigour.

Manurial treatment immediately suggests itself as another means to the same end. Frew (2), dealing with a somewhat similar problem, found superphosphates to be the best accelerators for the growth of barley. Heavy applications of nitrogenous manures, it is well-known, produce a "lush" growth in barley, and observations show that crops which have received such applications are particularly liable to show severe *Helminthosporium* symptoms.

That conditions which influence the *number of tillers* produced by barley plants, conditions such as *manuring* and *density of sowing*, certainly have an effect on the incidence of the disease in a crop, follows from what has been said in earlier paragraphs concerning the escape of tillers, in particular from the statements concerning the superior chance of the later tillers escaping.

Factors such as these which I have just mentioned, which, unlike the soil-temperature factor, do not affect the proportion of the crop which initially becomes infected, but influence the proportion of the attacked individual which becomes diseased, may be termed minor factors. In actual practice these minor factors would probably be ignored, but it is not my opinion that farmers can afford to ignore the one thoroughly important result of this study of conditions, namely that *winter-sown barley in Britain is particularly liable to be attacked.* Special care in the disinfection of the seed is therefore essential. Even though this disinfection should fall short of the ideal, the farmer is entitled to expect, according to the new attitude very much more than according to the old, that good husbandry, producing vigorous plants, will counteract the effect of bad seed.

19. Summary.

The accepted view has been that this fungus, in causing Leaf-stripe Disease, behaves as many Smut-fungi do, the mycelium spreading from the apical growing-point of the stem to the leaves and other parts.

It is *here claimed* that the leaves are first infected, and the stem-apex infected only in the final phase of the disease. Infection of the stem-apex is not a necessary preliminary to the production of a fully diseased plant. In fact, infection of that apex is, very often, followed swiftly by the death of the plant. This fungus cannot live as a "tolerated symbiont" in such growing-points.

For the proof of this main contention, as well as for the general increase of knowledge, stages in the life-history of affected plants are reviewed in turn.

The sources of infection for the germinating grain are described.

This is followed by evidence, revealed by the microscope, concerning the penetration from these sources, and the subsequent spread of the fungus.

The normal sequence of leaf symptoms, as also deviations from this sequence, provide useful evidence concerning the main thesis. By discussion of these (and of questions concerning the tillers) it is hoped that identification of cases of this disease is made easier.

Hitherto it has been considered that only while it remains outside the germinating seed is the fungus in an insecure position, at the mercy of circumstances such as temperature. The real importance of temperature at that time is here strongly reaffirmed, but, further, it is pointed out that the insecurity of the fungus in a Leaf-striped plant persists for a very much longer time. Vigorous shoots often grow away from the fungus. In this way, and in others, good farming has its reward in fighting this disease.

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ON THE STEM ROT OR WILT DISEASE OF CARNATIONS[1]

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(With Plate XIII.)

CONTENTS.

1. Introduction.

Losses among carnation growers due to a disease known as "stem rot" have only reached serious proportions since the War, although in other countries much trouble of a similar nature has been experienced for many years. At the instigation of the Director of the Royal Horticultural Society's Garden, Wisley, the author undertook an investigation with

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the object of finding out (1) whether or not the disease occurring in English nurseries is the same as that recorded elsewhere, (2) under what conditions the disease occurs and is spread, and (3) what control measures could be recommended to carnation growers.

2. Brief Review of Previous Work on Carnation Wilt Diseases.

The tree or perpetual flowering carnation was originally raised in France about the year 1840 but was later introduced into America and has since become known as the American carnation. It is from the latter country that the great majority of stocks grown in England have been derived.

In 1897 Sturgis (1) described a carnation disease generally known as "die-back" or "stem rot" the symptoms of which are very similar to those noted in the present investigation. Sturgis isolated a Fusarium which he did not name, but which was capable of bringing about similar symptoms when introduced into sterilised soil. He also showed that the disease could be spread by infected cuttings.

In 1898 Stewart (2) showed that Sturgis was probably dealing with two distinct diseases of which the "stem rot" was due to Rhizoctonia and involved a sudden wilting, and the "die-back" to a Fusarium which acted relatively slowly.

In 1900 Delacroix (3) published the result of a full investigation of a similar wilting disease which had caused considerable losses to growers in the south of France. He attributed the disease to a Fusarium which forms chlamydospores under certain conditions and which he named *Fusarium dianthi* P. and D. In a paper closely following this, Mangin (4) gave reasons for not accepting this name and stated that the Fusarium concerned was that hitherto known as *Fusarium roseum* Link.

In 1915 van der Bijl (5) investigated what is known as "wilt" or "crown rot" of carnations grown in the open in South Africa (Natal). This was of the sudden wilt type described by Stewart (2), involving a soft rot of the cortex at the base of the stem the xylem of which was stained brown. He isolated a Fusarium which on inoculation produced the disease and which in pure culture gave rise to a yellowish colour rather than a pink or rosy one and formed chlamydospores readily. Van der Bijl also showed that high temperatures and much moisture were favourable to the incidence and spread of the disease.

In 1920 Small (6) investigated a wilt of carnations and other plants in Uganda exactly similar to that described by van der Bijl in South Africa. A Fusarium was isolated the growth characters of which were in close

agreement with those given by van der Bijl, and which in a subsequent paper Small(7) gives reasons for identifying as *Fusarium udum* Butler, the cause of pigeon pea wilt in India.

3. Occurrence and Symptoms of the Disease in Carnation Houses.

The disease is most in evidence during the months of June, July and August when the general average temperature is higher for a longer period than at any other time of the year. Diseased two and three years old plants are then most conspicuous; but it is not uncommon for 18 months old plants and even cuttings, freshly transplanted, to show signs of wilting due to disease.

A characteristic symptom is the slow withering with loss of colour of the shoots one after another. The green colour gradually changes to a pale straw yellow and serves to distinguish such plants from others which wilt more suddenly from some other cause such as wireworm damage. If allowed to remain in the beds the wilt progresses until every part above ground is affected. The roots decay and under moist conditions the cortex of the collar becomes soft and rotten and bear pustules of Fusarium spores. The name of stem rot commonly employed by growers is due to this symptom, which, however, in the author's opinion is by no means constant. The disease is primarily a wilt.

During this investigation another trouble was frequently encountered both in growers' houses and in the experimental house at Wisley. This was a form of die-back which is included here because it was found that the same fungi are involved in both stem rot and die-back. From time to time a number of plants were observed with one or more young shoots dying back from the topmost node. Investigation showed that these shoots had been "stopped" by having their upper portions "pinched out," and that a few weeks later they had commenced to die back. This process goes on slowly for many weeks and even months; but so far I have not found that the rest of the plant becomes affected, the dead portion extending no further than the junction with the larger branch or main stem.

On more than one occasion freshly struck cuttings from different sources have been observed with similar wilting symptoms (Plate XIII). A considerable number of such plants die if the conditions of moisture and temperature remain unaltered. When both are diminished a certain number recover and become healthy plants. Investigation showed that two species of Fusarium were involved, one of which was distinct from those concerned in stem rot and die-back.

4. Greenhouse Experiments and Observations.

In the course of the investigation a rather striking fact was once observed in a nursery situated on heavy clay, and as it was intimately connected with local conditions and the prevalence of disease in certain beds and even particular portions of a bed it is recorded here in some detail.

In one of the older types of houses a patch of wilted plants occurred in the middle of every bed. The patches together formed a straight line at right angles to the length of the house; upon enquiry it was ascertained that an underground stream ran in the same direction. The disease was always present in these spots the soil of which throughout the year was much wetter than elsewhere, thus indicating that amongst the conditions favouring the disease a relatively high moisture content was important.

In the experimental house an attempt was made to imitate this state of affairs by dividing an improvised bed into two parts by a concrete partition. One half of the bed was made up with very shallow soil, not more than 4 in. deep, while in the other half the soil was nearly 2 ft. deep. Both were planted at the same time with different varieties of carnations, one half bed being the duplicate of the other in this respect. The half bed with deep soil was over-watered, while the other half was kept as dry as was consistent with good growth. During the 18 months which followed two things became apparent: (1) that the plants on the shallow dry soil were not much less in stature than those in the deep wet soil; and (2) that a much greater number of plants succumbed in the latter than in the former. The beds were made up with ordinary garden soil to which no infectious material had been added.

The observations were considerably interfered with by the attacks of mice which gnawed through a number of plants in the wet bed and by an attack of red spider in the dry bed. In spite of these complications considerably more plants presented the characteristic wilting and yellowing symptoms of the disease in the wet bed than in the dry one. An examination of the wilted plants showed mycelium in the lower parts (see below) and from some of them species of Fusarium were subsequently isolated.

This experiment would seem to confirm the observation made above, viz. that the stem rot disease is more prevalent under relatively moist soil conditions.

Among many growers the opinion is prevalent that deep planting favours the disease and that plants with the upper portions of their roots exposed are not so liable to attack.

5. Examination of Diseased Plants.

Attention was concentrated upon the stem rot or wilt disease as being more serious than either die-back or wilting of the cuttings. Microscopical examination was made of plants of different ages and in different stages of attack. Moreover, by the courtesy of Dr H. H. Storey a consignment of plants received from Natal was also examined.

In the English material mycelium was found in the stems just above ground level and in the upper parts of the roots of plants having only a few wilted shoots. The most satisfactory stain for revealing the presence of mycelium was found to be "Cotton Blue" dissolved in lactophenol, applied in the manner described by Klebahn (8). The collar of the plants contained more mycelium than any other part and nearly all its tissues from the epidermis to the pith were invaded by intra- and extra-cellular hyphae. Above and below this region the hyphae were more abundant in the xylem than in the other tissues, while at the very edge of the infected parts only a few hyphae could be seen in the vessels. It was a little surprising to find mycelium nearly an inch from the crown in the xylem of roots which appeared outwardly quite healthy.

Another striking fact observed in connection with the mycelium was the large amount of a gum-like substance in both tracheids and vessels, generally in the neighbourhood of the hyphae. In fact, in the roots and collar the presence of this substance, conspicuous in longitudinal sections, almost invariably indicated the position of the hyphae, some of which were actually within the gum-like mass. The majority of these embedded hyphae consisted of dead and empty cells, but a few retained the deep blue of the stain and had the appearance of being alive.

In tracing exactly how far the mycelium had reached and to what extent the actual wilting of the shoots was due to its presence, the bases of wilted shoots were examined just beyond their insertion with the main stem, but in no instance was mycelium found in such parts. On the other hand, much of the xylem was blocked up with the gum-like substance mentioned above.

6. The Isolation of a Number of Fusaria from Diseased Tissues.

By planting pieces of tissue taken from diseased zones containing mycelium on to agar plates Fusarium was almost invariably obtained. A large number of isolations were made from fresh material derived from various sources and were kept in pure culture on various agar media such as Dox's, potato broth and artichoke (see below).

From some wilted cuttings a strain of Fusarium designated *F.* 1 was isolated. From young wilted plants sent to the author a Fusarium, *F.* 2, slightly differing from the above in growth characters was obtained. This strain after a few months in pure culture on Dox's agar suddenly produced two sectors in a Petri dish with rosy pigmentation, one of which was isolated as *F.* 3. As however, subsequent inoculation experiments indicated that neither *F.* 2 nor *F.* 3 was parasitic they were discarded. Another strain, *F.* 4, was isolated from the dead portion of a stem of a still living plant. A fifth strain, *F.* 5, was obtained from two young diseased plants in the experimental house at Wisley. One of these showed typical stem rot lesions at the collar and had practically no roots when lifted. Pieces of the collar when kept over night on damp filter paper developed salmon coloured spore masses from which isolations were made. In the other plant the disease was of the die-back type and the fungus isolated from the wilted shoot proved to be *F.* 5.

Finally in July 1927, from plants 18 months old, obtained from a nursery in Kent, showing unmistakable signs of the wilt form of disease, *F.* 6 and *F.* 7 were isolated. These subsequently proved to be identical.

The material received from Natal was in too mouldy a condition for an adequate examination to be made; but in one plant the grey-brown streak in the main stem observed by van der Bijl(5) and Small(6 and 7) was found. No Fusarium however, was isolated from this plant but only a species of Verticillium which proved to be non-pathogenic.

7. Inoculation Experiments.

Inoculation experiments were carried out with the six strains mentioned above. Plants 12 or 18 months old were generally used for this purpose, but some recently struck cuttings were also inoculated. The inocula consisted of either a suspension of conidia in sterilised distilled water or mycelium plus a little agar. In the earlier inoculations the inocula were introduced into wounded internodes or leaf axils, or the "pinched out" internodes of young and vigorous shoots. In later experiments wounded older branches and the main stem at the collar were inoculated. The wounds were afterwards covered with cotton wool or tinfoil. One series of inoculated plants was kept in a cool greenhouse, another in a corner of the orchid house and a few in a glass incubator made to the author's design and kept in a large bay window of the laboratory.

The following are selections of the inoculations made:

A. Inoculations with F. 1 (*F. avenaceum*, see below). A young healthy plant having five upright shoots the tops of which were "pinched out" by hand was inoculated with spores and mycelium as follows: On 5. xi. 26 all the exposed nodes received a drop of distilled water. To two of them one loopful each of a rich spore suspension was added, two more received a small piece of mycelium on agar, and the fifth served as a control. The five shoots were then covered with tinfoil. The plant was placed in a

cool greenhouse. On 16. xii. 26 a very slight discoloration was observed below the tinfoil covering one of the inoculated shoots, the rest showed no change, and no further development took place.

Using the same inocula two similar plants were inoculated through wounds at the base of the main stem; the plants remained quite healthy.

A similar plant was treated as the plant first inoculated, but was kept in the laboratory in the sink and covered with a bell jar. In a few days a rich white growth developed over the wounded shoots which at the end of a month had died back to the next node. The atmosphere under the bell jar was fairly warm (25° C.) and very moist.

It was concluded from this and similar experiments that *F.* 1 at ordinary temperatures was not parasitic, although at relatively high temperatures and in a moist atmosphere, such as sometimes obtain in propagation houses, this Fusarium is probably capable of causing some damage.

B. Inoculations with F. 2, F. 3 *and* F. 4. Under ordinary conditions inoculations with these strains had no effect so they were discarded.

C. Inoculations with F. 5 (*F. herbarum*, see below). On 26. i. 27 pieces of mycelium of *F.* 5 were introduced into four slightly wounded internodes. A fifth internode wounded in the same way was not inoculated. All were covered with tinfoil, and the plant placed in the cool greenhouse. After eight days the first definite sign of infection was observed on one of the four inoculated shoots. A slight change of colour to a paler green than normal was quickly followed by very slight withering and shrivelling, the tips of most of the leaves exhibiting a few longitudinal depressions or striations due to contraction. On 9. ii. 27 another inoculated shoot exhibited similar symptoms, and on 15. ii. 27 a third shoot showed signs of infection. This shoot had been inoculated much further down into more woody tissue. The shrivelling of the leaves above the inoculated wound commenced at the base and spread towards the tips. On 19. ii. 27 the fourth shoot inoculated low down like the preceding became infected. On 17. iii. 27 all the inoculated shoots were dead, shrivelled and straw coloured above the wounds. The infection did not spread downwards past these places and the control shoot remained healthy.

On 21. i. 27 using a rich suspension of spores from a culture of *F.* 5 a plant was inoculated in four places, viz. on the tops of two "pinched out" shoots and into two wounded leaf axils. All were covered with tinfoil and the plant kept in the glass incubator at a temperature of about 24° C. On 3. ii. 27 one of the inoculated shoots wilted and withered, and after 16 days all four shoots showed signs of infection and subsequently withered. In none did the infection travel down the stem.

On 7. iii. 27 six shoots of a well grown plant were inoculated through incisions and afterwards covered with tinfoil. Two of the shoots were inoculated with mycelium and one with spores, both grown at 24° C. The other three received spores or mycelium grown at a temperature of 27°–30° C. No infection took place with either spores or mycelium produced at the higher temperature, but the other three shoots became infected in from five to nine days. From these and similar experiments it was concluded that *F.* 5, which had been originally isolated from the die-back form of disease, was an active causal agent of the disease which was involved under conditions of moderate temperature (24° C.) and moisture.

D. Inoculations with F. 6 *and* F. 7 (*F. culmorum*, see below). On 12. vii. 27 a large plant was inoculated in the following manner: the upper portions of three stems

were cut off and the stumps moistened with drops of distilled water. On one cut surface a little mycelium of *F.* 6 was placed, on another the spores from a culture of *F.* 7 were added and the third stem served as a control. The plant was stood in a dish containing a little water and the whole was covered with a bell jar in the laboratory at a temperature of about 26° C. A pink and white mycelium rapidly developed over the inoculated shoots but not over the control. By the end of August about three inches of each inoculated shoot had died back. On removing the bell jar and placing the plant in the cool greenhouse the die-back ceased.

A small seedling plant about six inches high was inoculated at soil level through a slit in the stem with mycelium from *F.* 6. The slit was pressed together and kept closed by damp soil. The plant was placed in a sink in the laboratory under a bell jar, which was removed after one month. On 8. viii. 27 the lowest leaves had turned yellow and there were slight signs of wilting above. On 15. viii. 27 the lowest leaves had become brown and shrivelled and the wilt above was now quite definite. On 30. viii. 27 the condition of the plant resembled that seen in growers' houses.

A similar seedling plant treated as above but placed in the orchid house and not under a bell jar was not infected.

A similar plant treated in the same way but in the cool greenhouse was not infected.

The above series seems to indicate the importance of moist conditions at the place of inoculation for infection to take place.

On 30. vii. 27 two three-year old plants, both with several upright branches, were inoculated with conidia from *F.* 7 by introducing a loopful of a suspension of spores into wounded leaf axils. Five leaf axils of each plant were slightly wounded with a sharp sterilised scalpel, and, of these, four on each plant were inoculated and one on each was left as a control. After inoculation each wound was tied round with one twist of thick string. Both plants were placed in the orchid house. Definite signs of infection were noted on 8. viii. 27 which became more pronounced on 11. viii. 27; by 30. viii. 27 two to three nodes below each inoculation had been killed. The controls remained healthy. This experiment shows that *F.* 7 (*F. culmorum*) is able to produce the die-back type of disease.

On 11. viii. 27 a three-year old plant was inoculated with conidia from *F.* 7 introduced as a rich suspension into a deep incision at the base of the main stem. The incision was closed by one twist of thick string and the plant placed in the orchid house. After 19 days much of the foliage was wilting and pink spore pustules were observed at the edge of the wound. The plant became completely withered in about a month. *F.* 7 was re-isolated from this plant.

On the same date a similar plant treated in the same way but kept in a cool greenhouse did not become infected.

On the same date a one-year old plant treated in the same way was placed under a bell jar in the laboratory. Three months elapsed before there were any signs of wilting, but by December the plant was dead.

On the same date a similar plant treated in the same way but kept in the orchid house was dead after four months with the stem rotted through at ground level.

This series not only indicates the necessity for moisture at the place of inoculation but that older plants are more rapidly affected than young ones. Furthermore, *F.* 7 is capable of producing the stem rot type of disease.

On 17. viii. 27 the soil around two young seedlings in separate pots, raised from sterilised seed in sterilised soil, was moistened by pouring 10 c.c. of a suspension of *F.* 7 conidia down the collar of each plant. One was kept in the cool greenhouse and the other in the orchid house. Both were killed by December. This again indicates that young plants take longer to succumb to the disease than older plants.

On 14. ix. 27 a three-year old plant was inoculated at the base of the stem about 1½ in. from the soil through a deep slit with conidia of *F.* 6. An inverted waxed paper cone was fixed round the stem at soil level and contained water in order to keep the inoculated place moist. The plant was kept in the laboratory and well watered. On 5. xi. 27 there were slight indications of infection and the plant was moved into the greenhouse on 17. xi. 27. The paper cone was kept filled with water the level of which did not quite reach the inoculated wound. By January 1928 the whole of the aerial portion had wilted and withered. In this instance infection first became evident after an interval of about seven weeks. A similar plant without the paper cone kept in the greenhouse the whole time was not infected. The infected plant was then examined. The portion above the wound was dead and pink spore pustules were present around the edges of the cut. The plant, however, remained firm in the ground and the roots were not infected at the time the examination was made. Abundant mycelium was found in all tissues except the pith between soil level and the wound. The cortex of this region was soft and rotten and resembled a case of typical stem rot.

On 1. xi. 27 a couple of two-year old plants were inoculated with conidia through incisions at the base of the stems about one inch above soil level. Each wound was wrapped round with thick string one end of which dipped into a vessel containing water. In this way the wounds were kept continually damp without the exclusion of air. Both plants were kept on the laboratory bench in a good light. After three weeks a shoot of one plant commenced to wilt. The youngest leaves did not open out but remained clasped within the older ones, which became paler in colour. Striations soon appeared upon the leaves which finally shrivelled. After six weeks from the start all the shoots of both plants had wilted and withered but both remained firm in the soil. On 21. i. 28 both stems parted from their roots about ½ in. below soil level. A control plant remained unaffected.

On 17. xi. 27 a two-year old plant was inoculated at the base of the stem with mycelium from *F.* 6. The wound was covered with damp muslin and wrapped round with string the end of which dipped in water. On 21. xii. 27 one shoot wilted and dried up; shortly afterwards growth entirely ceased and the plant slowly withered. On 19. i. 28 the plant was lifted and examined. The roots were sound. A red discoloured area was present in the wood about the wound and a narrow strip of brown wood extended as far as the roots. Mycelium was present from the wound in the stem to the base of the first wilted shoot but was not abundant. Much gumming was found everywhere except at the collar (below the wound) and tyloses were numerous.

8. Conclusions drawn from Inoculation Experiments.

From these inoculation experiments the following conclusions were drawn:

(1) The carnation disease investigated by the author is not due to *Fusarium udum* which causes a similar disease in Africa (7) characterised

by sudden wilting, early death (within three weeks), and a dark discoloration of the wood.

(2) There are at least two Fusaria intimately concerned.

(3) One of these, *F.* 5, is a causal agent involved in the die-back type of trouble under conditions of high temperature and moisture, while the other, *F.* 6, under similar conditions will bring about both die-back and stem rot.

(4) A third Fusarium, *F.* 1, under the same conditions may be weakly parasitic to shoots.

(5) Plants 18 months to two years old are affected more rapidly with the wilting type of disease than are either younger or older plants.

(6) The Fusaria involved gain entrance through wounds.

(7) Both spores and mycelium of *F.* 5 produced at 27° to 30° C. did not bring about infection, but similar material grown at 24° to 25° C. was able to do so.

9. The Vitality of the Conidia of *F.* 6 and *F.* 7 (*F. culmorum*).

The following observation is of some interest as concerning the vitality of spores under adverse conditions. The spore suspension of *F.* 6 and *F.* 7 used in some of the inoculations and kept in watch glasses covered with a Petri dish were examined periodically for a period of three months. The great majority of spores sank to the bottom in a very short time but a few remained on the surface. Nearly all the latter germinated within 12 hours, but the former remained dormant and only germinated when laid on the surface of agar or tap water, or when they reached the surface owing to the evaporation of the water in the watch glasses. The loss from this cause was made good from time to time by the addition of a little tap water. After three weeks to one month it was found that the sunken spores had changed in appearance. The two terminal cells (the spores were mostly 6-celled) seemed to be empty and dead while the four inner cells had become swollen oval bodies with thick walls, still colourless and with granular contents. In some instances only one or two of the inner cells had behaved in this manner while all the remaining cells appeared dead and empty.

This is the only instance of the formation of resting cells or chlamydospores which the author has observed in this particular Fusarium which under ordinary conditions of culture did not produce such bodies. When placed on agar the thick oval cells germinated in the course of 12 hours or so and continued to do so up to the end of three months when the contents of the watch glasses became contaminatedwith other organisms.

The importance of this observation lies in the possibility of introducing the disease through a contaminated water supply as Bewley (9) has pointed out.

10. The Production of Toxic Substances by *F.* 6.

As wilting associated with more or less definite yellowing of the foliage has been shown in other diseases to be due to the presence of toxic substances in the transpiration current, Aster wilt (10), sleepy disease of tomatoes (11), and the silvering of foliage due to *Stereum purpureum*[1], attempts were made to ascertain if the same was true of the carnation disease. The following experiment was set up. Small flasks were filled with about 20 c.c. of the following liquids:

(*a*) The liquid from a culture of *F.* 6 grown in Dox's *solution*, filtered through a Chamberland candle.

(*b*) A similar portion of sterilised Dox's *solution*.

(*c*) The liquid from steamed carnation stems in sterilised water.

(*d*) The liquid from a culture of *F.* 6 grown on steamed carnation stems in sterilised water, filtered through a Chamberland candle.

(*e*) Tap water.

(*f*) Sterilised distilled water.

Into each flask two vigorous shoots about 8 in. high were placed. After four days the leaves of the shoots in (*a*) were slightly paler in colour and had wilted somewhat, and striations due to contraction were present at the bases of most of the leaves. No change in colour was observed in the shoots of (*b*) but the leaves had wilted to about the same extent and striations were present. A few striations were present on the leaves of the shoots in (*c*). The shoots in (*e*) and (*f*) were normal. After a fortnight the shoots in (*a*) and (*d*), both in Fusarium liquid, showed considerable differences as compared with the others chiefly in the *yellowing* and withering of the lower leaves and in more extensive shrivelling.

The first sign of the effect of the Fusarium-liquid was a slight change of colour, the affected leaves and stems changing from normal green to pale green and finally pale yellow. This was followed by shrivelling which in the leaves generally commenced at the base and progressed onwards to the tips. While the leaves were still only pale green slight longitudinal depressions were formed and increased in number until the leaves appeared to be striated.

[1] The author wishes to thank Mr F. T. Brooks for a verbal communication of this interesting fact.

Inoculated and infected shoots cut off in the pale green stage and placed in water partially recovered, but similar shoots in the pale yellow or straw coloured stage did not recover when so treated. Transverse sections of such stems fixed in alcohol showed that the chlorophyll tissue of the cortex had more or less completely collapsed into a series of ridges and depressions resting upon the collenchymatous cylinder of the stem. In some instances the chlorophyll tissue had been reduced to a brown strip of squashed cells bringing the unaffected epidermis almost against the collenchymatous cylinder. The chlorophyll had become disintegrated into an amorphous yellow mass.

This experiment appears to indicate that toxic substances *are* produced as the result of the presence of the mycelium within the xylem and are taken up in the transpiration current to the chlorophyll tissue which is thereby killed.

In another experiment cut shoots were placed in two test tubes, one containing water, the other being empty. The shoots in the latter *wilted* in a few hours, the tips bending over the rim of the tube. There was no visible loss of colour for 12 days by which time the stem and the leaves had shrivelled with the formation of longitudinal striations similar to those seen in infected shoots. Finally the shoots became completely withered and straw coloured and were then indistinguishable from withered infected shoots. The shoots in water remained practically unchanged. The experiment is an instance of a true *wilt* due entirely to the withholding of water and which is distinguished from the wilt of *infected* shoots by the absence of loss of colour as a *first* symptom.

11. The Growth of the Carnation Fusaria at Various Temperatures and their Behaviour on Different Media.

The Fusaria were grown mostly on Dox's and potato broth agar. Other media such as steamed carnation stems in sterilised water, artichoke agar, Brown's asparagin agar and sterilised plugs of potato or carrot were also employed. On all except that of asparagin agar both *F.* 5 and *F.* 6 produced abundant aerial mycelium, mostly white, but sometimes streaked with pink or yellow, or both, with a characteristic rosy tint in the substratum, most marked on artichoke agar, and least so on Dox. On artichoke agar the colour was so intense that the glass itself was slightly tinted and remained so after washing in hot water. *F.* 1 did not produce any colour in the substratum of any of the media, but much white aerial mycelium. Salmon to orange coloured spore pustules were

formed after about a month on most media with the exception of asparagin agar on which the growth of all was relatively feeble.

A series of incubators ranging from 15° C. to 40° C. and varying by 5° C. was placed at the disposal of the author by the Imperial College of Science, by means of which the optimum and maximum temperatures for growth of these Fusaria were determined. A double series of plates containing Dox's agar inoculated with the Fusaria was run for about a fortnight and repeated two or three times. The increase in the average diameter of the growth measured daily was taken as indicating the daily rate of growth at each temperature (Brown (12)). The analysis of the figures so obtained showed that the optimum temperature for growth of the three Fusaria is the same, *i.e.* about 25° C. and that the maximum for all three lies between 35° C. and 40° C.

A further series of incubators run with differences of 2° C. between 15° C. and 30° C. and between 34° C. and 40° C. indicated that the best temperature for growth was about 26° C. and the maximum about 38° C. for the three Fusaria. Mr F. T. Brooks kindly made the necessary arrangements for growing the same Fusaria at the Cambridge Low Temperature Research Station in which inoculated slants of Dox's agar were placed at − 6° C., − 1° C. and 0° C. to + 1° C. These tubes were examined after an interval of six months when it was found that no growth had taken place at − 6° C. *F.* 1 (*F. avenaceum*) had produced much aerial growth and spore pustules at − 1° C., while the other two had not grown at this temperature. All had produced very appreciable growth and spore pustules at 0° C. to + 1° C. *F.* 5 and *F.* 6 formed a rosy tint in the substratum at this temperature.

The three Fusaria were also grown on Dox's agar slants rendered acid, neutral and alkaline by the addition of either sulphuric acid or caustic soda and brought to a definite *p*H value by means of the colorimetric method. On the acid side the *p*H value was 3·6 and on the alkaline 8·8. By the addition of two drops of universal indicator to the tubes while still hot, shaking and then allowing them to set, the changes of colour (and therefore of *p*H) could be observed during the growth of the fungi when compared with control tubes. The acid medium was pale pink and on it the three Fusaria grew very slowly at first. The colour of the medium gradually changed to pale blue, but not so blue as was the alkaline medium. Both *F.* 5 and *F.* 6 grew best on the neutral medium which also became slightly alkaline as indicated by the appearance of a very pale blue tint. On the alkaline medium the growth was a little less vigorous and the colour changed slightly towards the neutral. *F.* 1 grew best on

the alkaline medium the colour of which turned to rather a deeper blue than that indicated by the control tubes, and on the acid and neutral media the same tint was finally produced.

From these observations it was concluded that *F.* 1 prefers a more alkaline medium than do either *F.* 5 or *F.* 6 which grow best on the alkaline side of neutrality. At the time these cultures were made the identity of the Fusaria was not known and the writer was much interested to receive Dr Wollenweber's determinations, for the observations are in close agreement with those of Lundegärth (13) who happened to work with the same three species (*F. avenaceum*, *F. herbarum* and *F. culmorum*).

12. The Identity of the Fusaria Isolated from Diseased Carnations.

The author is fully conscious of the difficulties in naming Fusaria, particularly in view of the work of Brown (14) and Hansford (15), and of his own experience of the appearance of saltants in cultures of *F.* 2 (see above). Cultures were sent to Dr Wollenweber at Berlin who was kind enough to name the three Fusaria as follows: *F.* 1 = *Fusarium avenaceum* (Fries) Sacc., *F.* 5 = *Fusarium herbarum* (Corda) Fries, and *F.* 6 and *F.* 7 = *Fusarium culmorum* (W.G.S.) Sacc.

In letters to the author Dr Wollenweber pointed out how very widespread these three species were in Nature and that they occurred on a large number of plants belonging to different families; further, that *F. avenaceum* and *F. herbarum* could be regarded as varieties of the same fungus, so hard was it to distinguish them on morphological grounds.

For biological reasons however, as the present work shows, they can be fairly easily separated, for *F. avenaceum* is a saprophyte at ordinary temperatures, does not produce pigment on Dox's agar and grows well at − 1° C., whereas *F. herbarum* is a wound parasite on carnation shoots, produces a rosy colour in Dox's medium and will not grow at − 1° C.

A considerable number of more or less well defined species of Fusarium have been recorded as occurring upon carnations, a list of which is given by Wollenweber in the new edition of Sorauer's *Handbuch der Pflanzenkrankheiten*. Lewis (16), also quoted by Wollenweber, was able to produce a rot of carnation buds with a number of species none of which had been isolated from carnations but which had been obtained from apples, grasses, etc.

According to Wollenweber, the species which Delacroix (3) worked with and called *F. dianthi* P. and D. may have been *F. aurantiacum*

(Lk.) Sacc. (Elegans group), which is also very widespread on many different plants.

It seems clear from previous investigations (7 and 16) and from the present work that there are a number of more or less well defined species of Fusarium, widely spread in Nature, which under certain conditions can invade and kill the stem tissues of carnations.

13. The Conditions under which *F. herbarum* and *F. culmorum* can bring about Infection and the Propagation of the Disease.

The present investigation indicates that the presence of a relatively high moisture content of both soil and atmosphere, and a relatively high temperature (24°–26° C.) are important factors in relation to infection, and that these fungi can only gain entrance through wounds.

Lundegärth (13) has shown that a high (3 to 7 per cent.) concentration of carbon dioxide in the soil favours the growth of these Fusaria and leads to the attack of wheat seedlings. The very general occurrence of *F. avenaceum* in most soils is due, according to Lundegärth, to its tendency to produce a slightly alkaline reaction (see above), and the development of plant diseases in which Fusaria are concerned is favoured by plentiful applications of organic manures whereby the carbon dioxide content of the soil is increased, thus favouring the growth of these fungi.

The die-back type of the disease (in which "pinched out" shoots are involved) can probably be found in any nursery where the watering is rather overdone during the summer months, as both *F. herbarum* and *F. culmorum* are probably present in the soil of the beds at the very start, and both form spores on dead and decaying material. Infection is almost certainly due to the splashing of the spores on to the "pinched out" internodes during watering.

The exact place of infection in the stem rot form has not been definitely ascertained, but it is certainly through wounds due to various causes such as wireworm attack, and the natural cracking of the cortex of the collar of some varieties (Plate I). It is possible that *F. culmorum* may commence its attack as a die-back of one or more "pinched out" shoots and then pass down to the older parts of the plant, ending up at the roots. The experiments recorded here, however, do not support this view as in no instance of experimentally produced die-back did the stem rot form result. It is of course possible that the somewhat greater care with which the relatively few plants at Wisley were cultivated as compared with the many thousands in any nursery may have had something

to do with the possible arrest of the die-back form of disease. In support of this, attention may be called to the incidence of disease in the over-watered bed in the Wisley experiments and the absence of naturally occurring disease in potted plants in the same house. Here, the two factors of ventilation and moisture are involved as the pots were spaced at much wider intervals than were the plants in the beds which were the usual 8-in. apart either way. There are some varieties of carnations very prone to the stem rot disease, which when grown in contaminated soil are very liable to become infected. But besides the planting of susceptible varieties in soil already containing *F. culmorum* there is the additional risk of actually carrying the parasite along with its host when young plants are sent from one nursery to another. To save freight charges and space, plants as small as possible are transported and are generally recently potted up cuttings which have only been knocked out of their pots ("sixties") and are sent with the soil still surrounding the roots. Should this soil contain traces of *F. culmorum* it is almost certain to infect that of the new nursery, though infection of the plants probably does not take place, as has been shown, until the plants are much older.

Bewley (9) has shown that some water supplies contain Fusarium and other spores which are capable of introducing disease into nurseries, and the author has demonstrated that *F. culmorum* can form dormant cells when kept in water, which will germinate after three months.

14. Suggestions for the Control of the Disease.

As the three Fusaria concerned are widespread and probably occur in most soils, the best means of control is to sterilise the beds, preferably by steam, before planting. This, however, would be useless if cuttings were planted to the roots of which contaminated soil was adhering. The difficulty could be overcome in two ways: either by raising one's own plants, "struck" in sterilised sand; or by having a quarantine house in which all plants received from outside sources could be grown in sterilised soil for a period of at least two years.

Apart from soil sterilisation, every care should be taken in watering to avoid splashing the "stopped" plants and to keep the beds on the dry side rather than the reverse. Furthermore, the temperature should be kept as low as possible by ventilating and shading during the summer months. The possibility of introducing the disease through contaminated water should not be overlooked.

Among many growers the opinion is prevalent that deep planting is closely connected with the disease and that plants having the bases of

their roots slightly exposed are less often attacked. This may be connected with the natural cracking of the cortical tissues of the collar of certain varieties, but the point calls for further investigation.

15. Conclusions.

The chief conclusion to be drawn from the present investigation is that the English disease is not due to the same causal agents as in other countries, with the possible exception of the American die-back disease defined by Stewart (2).

The occasional wilt of very young plants in England is due to *F. culmorum* (W.G.S.) Sacc. The fairly common die-back of "stopped shoots" is due to *F. herbarum* (Corda) Fries and *F. culmorum* (W.G.S.) Sacc.; while the far more serious stem rot or wilt is due to *F. culmorum* (W.G.S.) Sacc., the only species so far met with in this type of disease.

The author would like to express his thanks to Prof. V. H. Blackman for advice and criticism throughout the investigation and to Dr Brown for his kindness in arranging for cultures to be grown at various temperatures at the Imperial College. To Mr F. T. Brooks his thanks are also due for kindly arranging to have cultures grown at the Cambridge Low Temperature Research Station. The author's thanks are also due to Dr Wollenweber, of Berlin, for naming the three Fusaria with which this paper deals.

16. Summary.

1. Previous investigations of carnation diseases, attributed to Fusaria, are briefly described and the scope of the present work is outlined.

2. The disease is most conspicuous in carnation houses during the height of summer and is characterised by a progressive wilting and withering of the shoots which eventually assume the colour of straw. Sometimes the cortex at the base of the main stem becomes rotten, but the disease is primarily a wilt. A die-back of "stopped" shoots was found to be fairly common.

3. Observations indicated the importance of much soil moisture as a factor in the incidence and prevalence of the disease.

4. Mycelium in the tissues of diseased plants was found mostly at the base of the stems, in all tissues, but was confined to the xylem above and below this area. In plants in the last stages of disease mycelium also occurred at the base of the shoots. The xylem of the upper portions of roots, outwardly healthy, was also found to be infected. Some hyphae

were seen to be embedded in a gum-like substance of which there was a great deal. The wood of recently wilted shoots contained much gum but no hyphae.

5. Isolations from diseased material gave Fusarium. The wilted cuttings yielded a strain of Fusarium, *F.* 1. *F.* 2 was isolated from young wilted plants and in culture produced a pink sector or saltant, *F.* 3. *F.* 4 was obtained from a similar source and *F.* 5 from two plants, one of which showed the stem rot type of disease, and the other the die-back form. *F.* 6 was obtained from the basal portion of an 18 months old plant exhibiting the wilting or stem rot type. *F.* 7, which proved to be identical with *F.* 6, came from the same source. Specimens from Natal only yielded a Verticillium which proved to be non-pathogenic.

6. Spores or mycelium were introduced into wounds in the nodes, internodes and collars of seedlings, 18 months and two years old plants under different conditions of temperature and humidity.

7. Under conditions of relatively high temperature and humid atmosphere, *F.* 1 can act as a weak parasite to shoots. *F.* 2, *F.* 3, and *F.* 4 proved to be non-pathogenic. *F.* 5 caused die-back only and *F.* 6 and *F.* 7 caused both die-back and stem rot. Plants 18 months to two years old are more rapidly affected than are either younger (seedlings) or older ones. The Fusaria can only bring about infection through wounds.

8. The spores of *F.* 6 germinate at once in drops of water or on agar, but sink to the bottom in water of any depth, do not germinate unless brought to the surface, and form a few thick-walled colourless resting bodies. These germinate within 12 hours when sown on agar, and such altered spores retain the power of germination for at least three months.

9. Experiments with filtered liquid in which *F.* 6 had been growing and in which carnation shoots were placed showed that a toxic substance was conveyed by the transpiration current to the chlorophyll tissues which were killed. No gumming was produced in such shoots and the first sign of wilting is a slight loss of colour produced in from four to five days. True wilting due to the withholding of water takes place in a few hours without loss of colour.

10. On solid media *F.* 1 produced abundant white aerial mycelium, while *F.* 5 and *F.* 6 formed white aerial hyphae streaked with pink and sometimes yellow, with always a rosy pink colour in the substratum.

The optimum temperature for growth was about 26° C. and the maximum about 38° C.

11. *F.* 1, *F.* 5, and *F.* 6 were identified by Wollenweber as *F. avenaceum* (Fries) Sacc., *F. herbarum* (Corda) Fries, and *F. culmorum* (W.G.S.) Sacc., respectively.

12. The conditions under which infection takes place are a relatively high temperature (24°–26° C.), high humidity and the presence of wounds. Deep planting also probably favours infection.

13. Sterilisation of the beds is recommended as the best means of control.

14. In England the occasional wilting of quite young plants is due to *F. culmorum* while the much commoner die-back of "stopped shoots" is caused by both *F. culmorum* and *F. herbarum*, and the more serious stem rot or wilt is due to *F. culmorum*.

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EXPLANATION OF PLATE XIII.

Wilting carnation cuttings with cracks at the collar.

(*Received October 19th*, 1928.)

DOWSON.—On the Stem Rot or Wilt Disease of Carnations (pp. 261–280).

THE INFLUENCE OF BRIGHT SUNSHINE UPON THE TOMATO UNDER GLASS

By W. F. BEWLEY, D.Sc.

(*Experimental and Research Station, Cheshunt.*)

(With 5 Text-figures.)

Glasshouse crops, in common with those grown in the open, vary from year to year in accordance with the weather. Some measure of protection is afforded by the screen of glass and artificial heat, but the necessary mechanical control of light, heat and humidity has not developed sufficiently to counteract external conditions. Some figures have been obtained at Cheshunt during the past eleven years, which suggest an intimate connection between the weight of the tomato crop and the amount of bright sunshine occurring during the season. They are considered of sufficient interest to warrant publication, although it is realised that many points require the confirmation which only prolonged investigation can provide.

The Relation between Crop Yield and Sunlight.

A definite fluctuation in annual yield was first noticed in 1920, when graphs were prepared from the records of the tomato experiments. The fluctuation was so striking that confirmation was sought on commercial nurseries in the district.

The graphs comprising Fig. 1 were prepared by plotting the years along an abscissa, and the crop weight in tons per acre along an ordinate. They illustrate the fluctuation in crop yield from 1917 to 1927 inclusive as occurring in four different nurseries *A–D* and in tomato house 2 at the Experimental Station. Details are given in Table II. Nurseries *A*, *B* and *D* are situated in the Lea Valley, and Nursery *C* near East Grinstead. It has been difficult to obtain records from nurseries because only blocks of houses which have received the same treatment over a period of years and in which the same variety had been cultivated during that time could be used. With one exception, the nurseries employed fulfilled these requirements, records being taken from blocks of houses about half an acre in extent. Nursery *B* was included because certain soil treatments produced differences which are readily observed.

Perhaps the most striking feature of the graphs in Fig. 1 is their approximate uniformity. Some differences are shown by Nursery *B*, where treatment of the soil with cresylic acid in 1920, and steam in 1922 and 1926, increased the yield. In 1923 an attack of *Verticillium wilt* conveyed in contaminated manure depressed the yield abnormally. The yield from the experimental house 2 was also increased in 1927 by steam sterilisation.

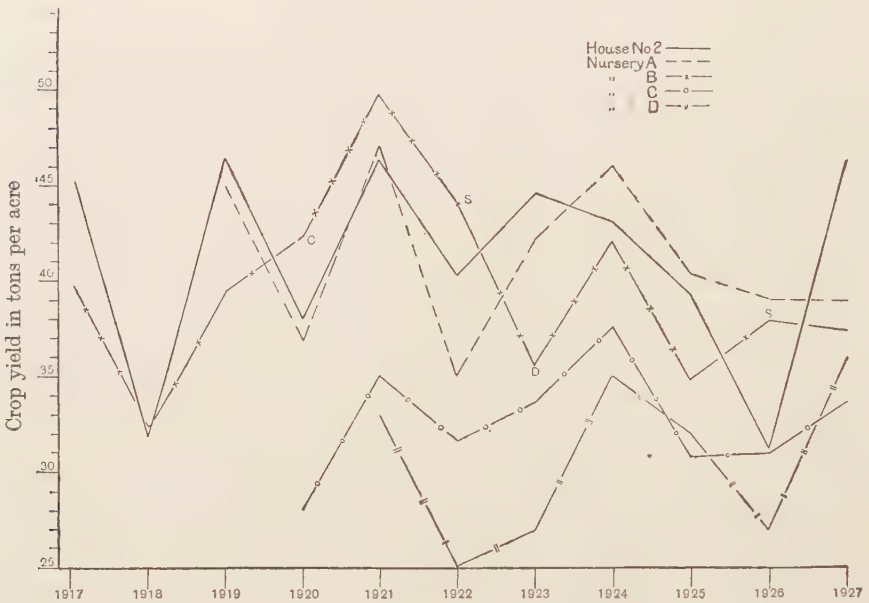

Fig. 1.

The peak year, 1921, suggested a reason for the variation in yield, because this year is remembered by all as one of exceptional sunshine.

Weather records for Cheshunt were obtained from the Meteorological Office, and those for the Surrey nursery from Mr W. G. Franks of the Observatory, Brockhurst, East Grinstead, to whom our grateful thanks are due. No figures for Cheshunt were available: they were calculated from those obtained at Rothamsted, Benington, Enfield and Tottenham, being the four nearest stations around Cheshunt. Details are given in Table I.

Fig. 2 depicts the curve of total hours of bright sunshine from April 1st to August 31st inclusive, this period being chosen because experience indicates that sunshine is especially valuable during these months. It is

less important during January to March when high temperatures stimulate leaf and stem development at the expense of the roots.

It will be seen that the sunshine curve follows the same trend as that

Fig. 2.

relating to crop yield with peaks in 1917, 1919, 1921 and 1924, and depressions in 1918, 1920, 1922, 1923, 1925, 1926 and 1927, the last being the exception at East Grinstead. The data from East Grinstead shows a

Fig. 3.

marked relationship between bright sunshine and crop yield. Similar results are described by Tippett[1] from observation of the wheat yields at Rothamsted.

[1] Tippett, L. H. C. "On the Effect of Sunshine on Wheat Yield at Rothamsted." *Journ. of Agr. Sci.* xvi, pt. 2, 1926.

Rainfall and temperature might be expected to affect the plant through its entire life. The graph of average maximum day temperature from January 1st to September 30th inclusive, and that for total rainfall during the same period are given in Figs. 3 and 4 respectively. It will be seen that, while these factors undoubtedly affect the total weight of crop produced, they are not so important as bright sunshine.

The Relation of Manurial Treatment to Sunshine.

The weather also exerts a strong influence on the type of growth—and for successful cultivation of a rapidly growing plant like the tomato under all conditions, the cultivator must know how to alter not only his temperature and humidity, but also his manurial treatment in accordance with prevailing weather conditions.

Fig. 4.

An attempt has been made to obtain some information on this point by studying the records of manurial plots in combination with meteorological conditions. In Fig. 5, the crop weight from four different plots during a period of eleven years is shown graphically, ordinary data being shown in Table III. The plots are situated in house 3, Series J, at the Cheshunt Station, and the treatment of each plot has remained constant since 1916. One plot has received complete artificials, and in three others, nitrogen, phosphates and potash respectively have been omitted

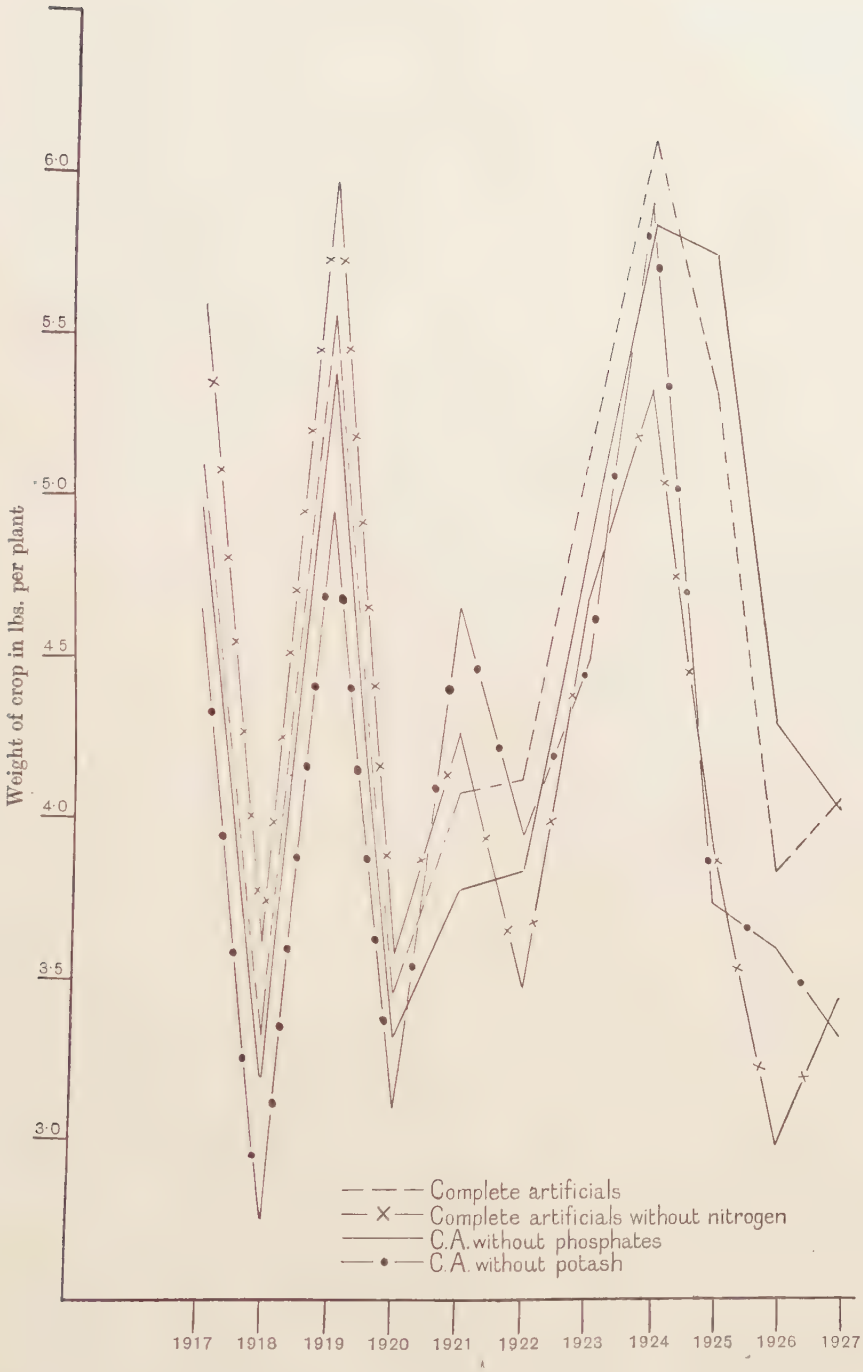
Weight of crop in lbs. per plant
6·0
5·5
5·0
4·5
4·0
3·5
3·0
1917
1918
1919
1920
1921
1922
1923
1924
1925
1926
1927
Complete artificials
Complete artificials without nitrogen
C.A. without phosphates
C.A. without potash

Fig. 5.

each year. The house was steamed in 1924, and succeeding years must be omitted from the present discussion.

Considering the years 1917 to 1923, the relation between crop yield and sunshine can again be noted. An interesting point is the uniformity of the graphs illustrating the different plots, up to 1921, when the complete artificials without potash plot, which had previously given the lowest yield, suddenly rose to be the highest. This at once suggests that prolonged sunshine has a similar effect on the plant to that produced by potash manures, the omission of which had previously caused a relatively low yield.

The practical conclusion from these experiments, first suggested in 1921[1], is that the tomato crop requires less potash in fine sunny summers than during dull weather when it is most valuable, and that the amount of nitrogenous fertilisers must be increased during sunny weather. Observation on commercial nurseries has confirmed this and improved crops have been obtained by manuring in accordance with these conclusions.

Summary.

1. The tomato crop produced in glasshouses is affected by prevailing weather conditions.

2. The yield per acre varies directly in relation to the total hours of bright sunshine during the period April 1st to September 30th.

3. Total sunshine also affects the potash requirement of the tomato, less potash being required during bright sunny weather than under dull conditions.

Table I.

Meteorological Data.

	Total hours bright sunshine, April to August inclusive		Average maximum day temperature, °F., January to September inclusive		Total rainfall in inches, January to September inclusive	
Year	Cheshunt	E. Grinstead	Cheshunt	E. Grinstead	Cheshunt	E. Grinstead
1917	963·9	—	54·1	—	20·5	—
1918	909·8	—	57·8	—	20·1	—
1919	971·9	—	55·5	—	19·1	—
1920	843·4	985·9	56·6	57·4	18·8	24·5
1921	1184·1	1289·0	59·8	61·0	10·7	13·6
1922	957·1	971·1	55·5	55·4	21·1	27·1
1923	911·0	1042·1	56·7	58·0	17·5	25·3
1924	972·3	1164·2	55·8	56·8	25·4	29·7
1925	908·8	1134·4	56·6	57·7	18·9	26·8
1926	838·2	1019·4	57·0	58·5	19·4	22·9
1927	788·1	1076·3	56·9	57·1	24·6	33·2

[1] *Seventh Annual Report of the Experimental and Research Station, Cheshunt*, p. 14, 1921.

Table II.

Tomato crop in tons per acre.

Year	Nursery *A*	Nursery *B*	Nursery *C*	Nursery *D*	House 2
1917	51·2	39·7	—	—	45·3
1918	52·5	32·3	—	—	31·9
1919	56·0	39·5	—	—	46·4
1920	54·1	41·3	28·0	—	37·0
1921	53·8	49·7	35·0	33·0	46·4
1922	46·0	44·0	31·6	25·1	40·4
1923	46·5	35·5	33·6	27·0	40·6
1924	46·3	42·0	37·6	35·0	41·3
1925	41·2	34·8	30·8	32·0	39·2
1926	40·2	37·8	31·2	28·0	31·2
1927	48·5	37·3	33·6	36·0	46·4

Table III.

Tomato crop in lbs. per plant from manurial plots.

Year	Complete artificials	Complete artificials (less nitrogen)	Complete artificials (less potash)	Complete artificials (less phosphate)
1916	4·45	5·40	5·40	4·50
1917	5·11	5·60	4·65	4·98
1918	3·32	3·62	2·76	3·20
1919	5·57	5·98	4·95	5·38
1920	3·45	3·57	3·11	3·33
1921	4·08	4·27	4·65	3·78
1922	4·13	3·47	3·96	3·85
1923	5·09	4·69	4·51	5·01
1924	6·12	5·34	5·92	5·87
1925	5·34	3·92	3·75	5·77
1926	3·84	2·98	3·61	4·31
1927	4·07	3·44	3·33	4·06

(*Received October 30th,* 1928.)

THE BIOLOGY OF THYSANOPTERA WITH REFERENCE TO THE COTTON PLANT

IV. THE RELATION BETWEEN THE DEGREE OF INFESTATION AND SURFACE CAKING OF THE SOIL

By ELSIE I. MACGILL, M.Sc.

(*University of Manchester.*)

(With 2 Text-figures.)

During the summer of 1926 experiments were carried out concerning the influence of the water supply of a plant upon the degree of infestation of that plant by *Thrips* (2). As a result of these experiments a suggestion was made that "one factor concerned is the influence of heavy water supply upon the texture of certain soils in promoting surface caking which will act inimically to soil pupating species of Thysanoptera"; and in 1927 it was decided to devote special attention to this point.

The variety of cotton used in the following experiments was the Webber strain of American Upland Cotton used in the second series of experiments in 1926 (2). Four blocks of cotton plants, *A*, *B*, and *X*, *Y*, were taken, each consisting of about 30 plants grown in pots of 23 cm. diameter and arranged as in the diagram (Fig. 1).

The shortest distance between any of the blocks of cotton was approximately 120 cm. and it has been shown in a previous paper (2) that at even shorter distances than this, there is very little migration of thrips from one block to another; any insects which did migrate were almost certainly adult thrips which had already oviposited; but for this reason, and also because the adult insect is much more active than the larva and is therefore more likely to be lost during the counts, the number of larvae per unit area of foliage gives a much more exact indication of the degree of infestation.

In all cases the soil—a medium clay loam which easily became caked—and the value of water supply—800 c.c. per pot per week—were the same, but in the case of blocks *A* and *B* the soil was never allowed to cake, the surface to a depth of 2–3 in. being kept in a state of fine tilth, while in blocks *X* and *Y* the soil was left undisturbed. The texture of the

surface soil in the pot did not seem to affect the health of the cotton plants, as blocks *Y* and *B* contained, on the whole, larger plants than the other two blocks. As in the previous season the plants were singularly free from infestation by insects other than thrips; apart from one or two plants slightly attacked by an aphid species, a few isolated individuals of

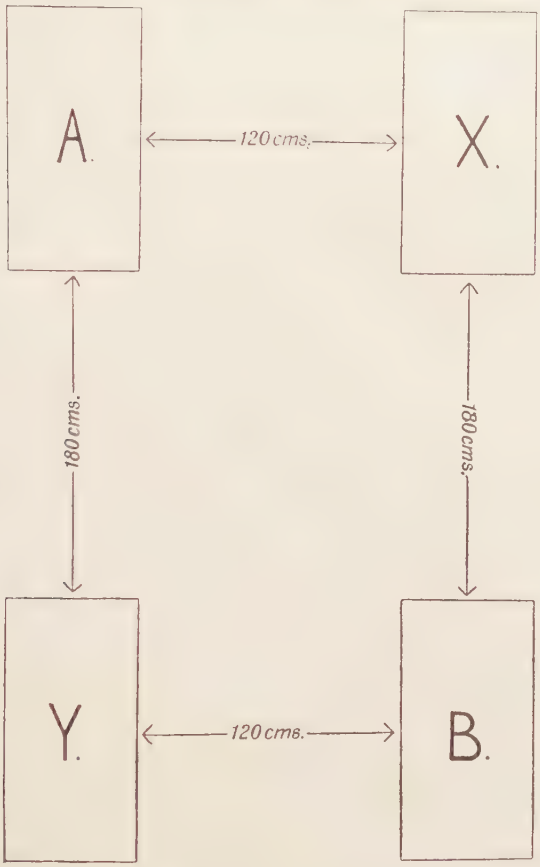

Fig. 1. Diagram showing the arrangement of the four blocks of cotton plants.

white-fly and leaf-hoppers were the only other insects noticed on the plants. Spiders and a species of predaceous mite occurred on all the blocks of plants but did not appear to be sufficiently numerous to decrease the number of thrips to any material extent.

Infestation counts of the thrips were made at intervals of a few days in the manner described in an earlier paper (2) and the infestation factor for each block thus obtained.

If the figures for the 1927 experiments are compared with those for 1926, two differences become apparent:

(1) The infestation factors for 1927 are considerably higher than the factors for 1926.

The highest factor for the whole period obtained in 1926 was 36·6 thrips per 100 sq. cm. of leaf surface, while in 1927 the highest factor was 65·3 thrips per 100 sq. cm., and three of the four blocks had an infestation factor greater than 36 thrips per 100 sq. cm. of leaf surface.

(2) On the whole the size of the leaves was less in 1927 than in 1926.

The second of these two differences can be explained by the fact that in the second series of experiments the plants were receiving a slightly smaller supply of water, so there was no tendency for the plants to form large leaves, as they do when the water supply is larger than necessary. The higher infestation factor in 1927 is explicable when a comparison is made of the temperature and humidity of the glasshouse for the two periods. In 1926 the mean temperature for the time during which counts of thrips were made was 19° C. (max. 26·7° C., min. 11° C.) and the mean relative humidity for the same period was 83·5 per cent. In 1927 the mean temperature for the corresponding period was 21° C. (max. 29·5° C., min. 11·9° C.) and the mean relative humidity 72·6 per cent., so that in 1927 the glasshouse conditions were a little more favourable for the multiplication of *Thrips tabaci* than in 1926 (1).

The mean infestation for each of the four blocks of cotton plants for the period during which counts were made was as follows:

A. 65·3 thrips per 100 sq. cm. of leaf surface.
B. 42·8 thrips per 100 sq. cm. of leaf surface.
X. 39·4 thrips per 100 sq. cm. of leaf surface.
Y. 21·6 thrips per 100 sq. cm. of leaf surface.

Except for a short time at the beginning of the counts, block *A*, which was one of the blocks in which the surface soil was tilled, showed decidedly the greatest infestation by thrips, while block *Y*, in which the surface soil was allowed to become caked, was the least infested of the four blocks. The other two blocks, *B* (tilled soil) and *X* (caked soil), did not show such a marked difference, but as *B* showed a slightly higher infestation than *X*, the result is in agreement with the former one. If *A* and *B* are taken together as one block, and *X* and *Y* together as a second, the difference in the degree of infestation by thrips between blocks of plants with the surface soil tilled and those in which the soil was left undisturbed, is very marked (Fig. 2).

Mean infestation factor of A and B—54 thrips per 100 sq. cm. of leaf surface.

Mean infestation factor of X and Y—30 thrips per 100 sq. cm. of leaf surface.

The difference between the infestation factors for the two blocks was almost entirely due to differences in the numbers of larvae on the plants, the respective numbers of adult insects obtained from each block only differing slightly; the mean adult infestation factor of A and B was

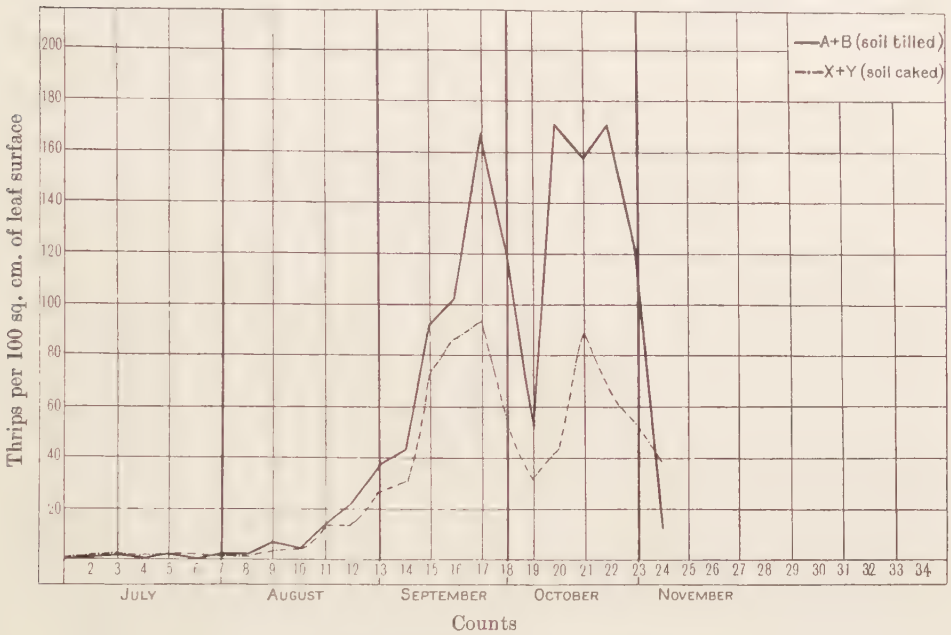

Fig. 2. The degree of infestation of two blocks of cotton plants; $A + B$ with tilled, and $X + Y$ with caked surface soil.

7 thrips per 100 sq. cm. of leaf surface, and the mean adult infestation factor of X and Y was 6·5 thrips per 100 sq. cm. of leaf surface, but it has already been pointed out that the adult infestation factor is very much less reliable than the larval one, as there is more likelihood of the adult insects being lost during the counts.

The highest infestation factor for adults plus larvae obtained during the counts was 255 thrips per 100 sq. cm. of leaf surface, which occurred on block A about the middle of October; the highest larval infestation factor (243 thrips per 100 sq. cm.) occurred on the same block at the same

time, but the highest adult factor, though occurring on the same date, was from block *X*, where 43·6 adults per 100 sq. cm. of leaf surface were counted.

The mean number of thrips per leaf for each block was:

A. 38 thrips per leaf.	*B*. 32 thrips per leaf.
X. 30 thrips per leaf.	*Y*. 24 thrips per leaf.

The largest actual number of thrips per leaf was counted at the end of September on block *Y*, when 347 thrips were obtained from one leaf, the largest number of larvae (328) was from the same block during the same count, though block *A* at the same time had one leaf with almost as many larvae (316). The largest number of adult insects (66) on a single leaf occurred on block *X* in the middle of September. The highest total number of thrips at any one count (10 leaves) was 1565 thrips from block *A* at the end of September—the largest number of larvae (1468) being on the same block at the same time; block *X* again had the largest number of adult insects per count, this was 267 thrips and was obtained in the middle of September.

The actual maximum number of thrips thus occurred at the end of September and after this there was a very decided fall in the number of insects. In October the glasshouse, which had not been heated artificially since the middle of July, was heated and shortly after this the number of thrips began to increase. When the figures for temperature and humidity were examined, it was found that after the glasshouse was heated the mean weekly temperature was slightly higher and the mean weekly relative humidity about 10 degrees less, so that the increase in the number of thrips seemed to be directly due to the higher temperature and decreased humidity, but on examining the graphs for the 1926 experiments, the same bimodal curves are found without any corresponding variations in the temperature and humidity. It seems probable, therefore, that the two peaks of the curve, especially as the time between them is approximately three weeks (1), simply represent succeeding generations of the thrips and that the fall in the number of insects is caused by the majority of them being at that time in the prepupal and pupal stages, and therefore not found on the plants. The earlier generations do not show so plainly on the graph, and this may be partly due to a lower post-reproduction mortality of adult thrips under the more favourable conditions in the early part of the season, and also because of the much smaller number of insects involved.

The results of these experiments agree with the conclusion arrived at

during 1926, that one effect of a heavy water supply is to promote surface caking of the soil, and that this is unfavourable to soil pupating species of thrips.

I should like to take this opportunity of thanking Prof. J. S. Dunkerly for his helpful criticism and advice, and also Miss A. O. Martin, M.Sc., for her assistance in counting the thrips.

SUMMARY.

1. Results of experiments on the effect of water supply on the infestation of a plant by thrips suggested that the surface caking of the soil has an important influence on the degree of infestation.

2. Plants grown in pots in which the surface of the soil was tilled showed a higher infestation by thrips than plants in similar pots, but in which the soil was allowed to cake, although both sets of plants were receiving an equal water supply, and all other conditions were the same.

3. The difference in the infestation factors for the two groups was largely due to differences in the numbers of larval thrips, but it is pointed out that the number of larvae forms a more reliable factor than the number of adult insects.

4. The present experiments support the suggestion put forward that surface caking of the soil acts inimically to soil pupating species of thrips.

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A RECORDING SCALE FOR BEE HIVES

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(With 4 Text-figures.)

The use of a hive on scales, the weight of which is recorded at least daily during the season, is strongly to be advised in any apiary. Unfortunately such a procedure is seldom adopted in this country. Continental bee-keepers, on the other hand, have their "Ruche sur bascule" or their "Wagstock," and many look upon it as part of the necessary equipment of the apiary. It enables them to keep an eye on the progress of the colonies and of seasonal effects thereon, and also serves as a guide in planning their work among the bees. Fortunately neither great sensitiveness nor extreme accuracy is essential for this purpose. The main object is to note the tendency of the weight changes, whether it be towards an increase or a decrease.

The most convenient type for general use is a platform scale large enough to take a single-walled hive of the dimensions used in the apiary, so that the centre of gravity of the hive may be over the centre of the platform. Both hive and scale should be protected from rain and from wind. Apart from the question of deterioration of the balance, due to exposure to the weather, it is surprising to note the weight of water added to a hive by a shower of rain. In windy weather it is difficult to weigh hives in the open because they sway about. The weight should be taken before or after the day's work, preferably before flying commences for the day. Readings taken at odd times of day may be very misleading. At the apiaries of the Dominion Experimental Farms of Canada, daily readings are taken at 7 a.m.

For the scientific investigator, greater sensitiveness is necessary, and some sort of recording mechanism is a great help. Mr C. B. Williams, formerly Government Entomologist at Cairo, has devised such an instrument (4). Recording scales have been used by Parks in Texas (3), and by the Bureau of Entomology at Washington. Williams used a platform scale having a pen on the end of the steelyard, which writes on a revolving drum such as is used in meteorological instruments. Employing

a really high-class scale, this arrangement should prove extremely useful for research work.

A recording balance, now in use at the Experimental Apiary at Rothamsted, although not so neat as the instrument to which reference has just been made, is giving satisfactory records, and has the advantage of being extremely sensitive. A simple scale beam *B* carries the hive and weights. Close behind this is a board, and mounted on a bracket is the clock drum *D* on which is the chart for the day. A pen *P* of the type generally used in meteorological instruments is fixed to an upright on

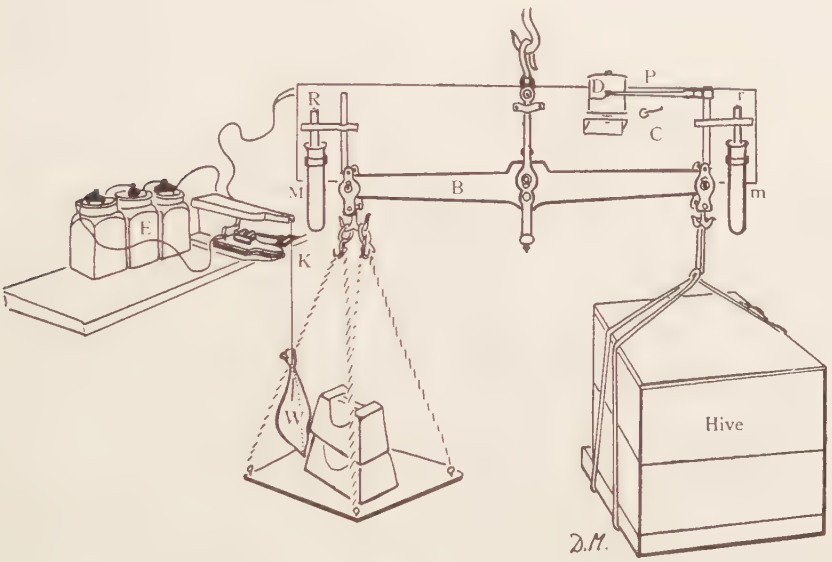


Fig. 1.

one of the stirrups of the balance, and records the changes of weight upon the drum. In order to steady the balance, two glass rods *R*, *r* are clamped in a vertical position at the ends of the beam. These dip in cups of mercury *M*, *m* supported on the board at the back. An upward pressure is exerted on the end of the beam, equal to the weight of mercury displaced by the rod. When the beam is inclined, the pressure is increased at the lower end, and correspondingly relaxed at the higher. By using larger rods in summer and smaller ones in winter, the range included on the chart can be adjusted to suit the expected variations in weight. (This principle is borrowed from Mr Williams' apparatus[1].)

[1] It has been suggested that two vertical helicoid springs in tension would serve the same purpose.

A device for automatically adding a weight has greatly extended the usefulness of this balance. A similar arrangement had previously been used by Oden (2) and Keen (1). When the hive increases in weight, say during a honey flow, the pen reaches the bottom of the chart and touches the contact wire *C*, completing an electric circuit. The mechanism consists of an ordinary electric bell from which the gong has been removed. A saw cut has been made in the hammer and the knife *K* (a safety razor blade) inserted. The weight *W* (a bag of shot) is suspended by a thread which passes within a fraction of an inch of the blade without actually touching it. When the circuit is completed the knife saws rapidly through the thread. The weight then gently drops on to the pan, the beam returns to the level position and brings the pen to the centre of the chart, ready to record a further increase. If necessary it would be possible to arrange for the addition of further weights in this manner, but one bag of four pounds weight has been found to give sufficient margin to allow the machine to be left unattended on Sundays and holidays.

A typical daily chart during the honey flow is shown in Fig. 2. (It will be seen that the record made by this instrument is inverted: a rise in weight being indicated by a downward line.) In the morning there is at first a drop in weight due to the exodus of bees. Later the hive is for a time in equilibrium, the outgoing bees being balanced by those that are already returning loaded. Then there begins a rise; the full field force is now at work and arrivals and departures are about equal in numbers, but the arrivals bring nectar water or pollen, and the outgoing bees are not laden. This state of affairs continues while forage is available. Up to the present, no very marked instance of plants yielding nectar in the morning or in the afternoon only, has been noticed in this district. Irregularities are chiefly due to threatened storms or to the sky becoming overcast. In the evening bees are returning and fewer are going out and there is often a tendency for the curve to become steeper at this time. When the last foragers are home the loss of weight due to evaporation and respiration becomes evident. This has been masked during the day by the changes due to other activities of the hive. Evaporation continues steadily through the night until bees begin to fly on the following morning.

Fig. 3 is a similar chart and shows a case where the automatic weight release has come into play, allowing the trace to continue on the same chart without the intervention of the observer.

Fig. 4 is a composite chart in which the Monday curve is made to follow on from that of Sunday. At 6.45 p.m. the weight was set by the observer. The evaporation of surplus moisture on the night of July 8th–9th is well

seen. There is quite a small drop which may perhaps be attributed to the earliest scouts leaving the hive. Normally this is followed by a general exodus as in the two previous examples. However, on three or more consecutive nights at about this time, a definite rise was observed (marked with an asterisk), which appears to be due to the return of bees which had been benighted on the previous evening and taken shelter in the fields.

Fig. 2.

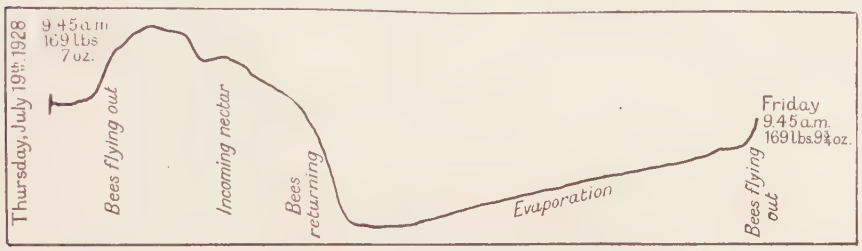
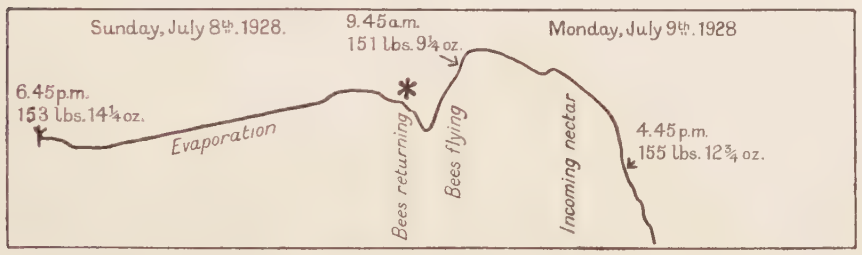


Fig. 3.

Fig. 4.



The moon was in the third quarter, the nights were warm and the bees were working white clover. It will be noticed that bees were working late on the previous night.

Many other facts are recorded on the tell-tale chart; as, for instance, when a mouse took its lodging among the quilts of the hive. On one occasion, after the honey flow was over, an unexpected increase in weight led to the detection of robbing from a neighbouring apiary. Wind causes much swaying even in the hut in which the instrument is housed. Fog

has sometimes been observed to cause an increase in weight during winter when no flying is taking place, owing to absorption of moisture by the combs and the wood of the hive. Distinct drops in weight during winter occur on those days when cleansing flights are taken, and these show a very definite relationship with the hours of sunshine recorded.

It has been suggested that some races of bees are early risers; that some work in unfavourable weather, while others are fair-weather bees, and that some work at certain sources of nectar neglected by others. Hives on recording scales, such as the one described, would be of great use in investigating these and other problems.

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A SURVEY OF THE INSECT AND OTHER INVERTEBRATE FAUNA OF PERMANENT PASTURE AND ARABLE LAND OF CERTAIN SOIL TYPES AT ABERYSTWYTH

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(With 4 Text-figures.)

CONTENTS.

1. INTRODUCTION.

The investigation described in this paper was carried out from January 1925 to February 1926. The fauna of four distinct soil types of a permanent pasture and one of an arable area has been considered. In order

to define as exactly as possible the conditions under which the soil fauna was existing in the areas examined, and for the sake of comparison with the results of previous workers, mechanical and chemical analyses of all the areas were obtained. For the data contained in Tables II and III (except moisture and loss on ignition in sample 1) I am indebted to the work of Mr R. O. Davies, M.Sc. I am also indebted to Prof. R. D. Laurie for general assistance rendered to me.

A botanical analysis of each of the four pasture areas was also carried out so that the relation of faunal and floral differences might be studied. Plant life, moreover, acts to a considerable extent as an indicator of the physical and chemical nature of the soil.

2. GENERAL DESCRIPTION OF THE DISTRICT.

The investigation was carried out at Nantcellan Fawr, the farm of the Agricultural Department, University College of Wales, Aberystwyth, which is situated at a distance of three miles to the north-east of Aberystwyth in the Clarach Valley, and about a mile from the coast. The locality had the advantage of not being near any industrial town, so that the effects due to atmospheric impurities were negligible. It may be considered as typical physiographically of at least the conditions which prevail throughout the Clarach Valley. The Clarach Valley is noted for its earliness in growth, maturity and ripening of crops, particularly the side with a southern aspect, on which the College Farm is situated. The rocks from which the soils in the Clarach Valley have been formed belong to gritty strata of the Ordovician and Silurian systems. The transformation into soil has been affected mainly through the disintegrating effects of mechanical forces, and not through a process of rock decay involving chemical changes which would have rendered the soil fundamentally different from its original structure. Most of the land of the College Farm possesses a southern aspect, with the result that it is well sheltered from the north and the north-east, but is exposed to the south. There is less danger from the high south-west winds, which are the prevailing winds of the district and are not infrequently in the form of gales, than from the north and north-easterly winds, as a cause of excessive evaporation of moisture from the soil. The action of the north and easterly winds which are often very prevalent in late spring and early autumn, has very often a highly destructive effect in many parts of Wales on soil life, causing rapid evaporation of not only the gravitational but the capillary water as well; the latter being at times so diminished after a period of drought that the consequences prove fatal. It is the unseasonable drying action

of these winds at these times of the year, when hibernating stages have not been reached, or have passed, that plays havoc with terrestrial insects.

The average yearly rainfall at Aberystwyth for the last thirteen years, which is not likely to differ appreciably from that at the College Farm, amounts to 38·5 in. The altitude of the area investigated varies from a few feet to about 300 feet.

With the exception of the low lying alluvial pasture of Cae'r Efail and the Boulder Clay area of Cae Mawr, the region under investigation is excellently drained, yet it is not very liable to suffer from drought. The Boulder Clay soils from their plastic nature are not easily affected, and although the Lighter Drifts show the effects of dry periods at an earlier date, these effects as a rule are not very marked.

The soils of the farm have been divided, according to their mechanical composition, into four distinct groups, viz. Alluvial soil, Heavier Drift soil, Lighter Drift soil, and Sedentary soil. A typical area for sampling was chosen from land which has been permanent pasture for the last forty years, from which each of these four soil types was obtained and for comparison a tract of arable land was selected, which has been under cultivation for an equally long period, and was of the Light Drift soil type.

3. DESCRIPTION OF AREAS EXAMINED.

The fields are variable in size and are usually separated by hedges of hawthorn, which often stand on a low bank, together with occasional blackthorn, hazel and furze bushes, and a few trees, the commonest being the ash and elm.

(*a*) Pasture Areas.

Cae'r Efail. This field shows two distinct soil types, an alluvial area and a lighter drift area. The possibility of deducing some important conclusions from an examination of their fauna was indicated.

Alluvial Area. Approximately fifteen acres in size, comprising the lower reaches of the College Farm, and skirting the northern side of the Clarach stream. It has an altitude of only a few feet, and receives the drainage waters from the higher lands on its northern aspect. During flood times the waters of the Clarach stream overflow on to this area, resulting in a water-logged soil, with an abnormal accumulation of organic matter.

Although the mechanical and chemical analyses of the soil of this area show a very high percentage of organic matter, yet the latter is not

sufficient to modify the heavy nature induced by the high proportion of clay and silt (see Table III under heading "Loss on ignition").

Lighter Drift Area. This consists of a narrow portion of about four acres on slightly higher ground than the alluvial area. Owing to its position, and the high percentage of the coarser particles, the soil is of a light open nature, and possesses an excellent drainage. It emerges indefinitely into the alluvial pasture below and, like it, has been under permanent pasture for many years. It has accumulated a large amount of organic matter, though naturally at a much slower rate than the low-lying alluvial soil.

Cae Mawr. The character of the soil in this field varies to a marked extent; that of the north-eastern portion contains a high proportion of clay and has been classified as a Boulder Clay area, that of the south-eastern portion is more sandy, whilst the area on the western side is a Sedentary soil.

Seeing that this field afforded at least two distinct soil types, a Boulder Clay area and a Sedentary soil area, and that the whole field had received practically the same treatment for a very long period, a difference in fauna might be expected to be due largely to the physical nature of the soil types.

Boulder Clay Area. Comprises about two acres of the north-east section of this field. On account of its tenacious character, with an underlying stratum of a heavy nature, which prevents leaching, it has a large retentive capacity for moisture and tends to become water-logged during wet weather. The disadvantage is not, however, very great owing to the drainage afforded by the slight slope towards the south and south-west, where it merges indefinitely into the Lighter Drift soil.

Sedentary Area. This narrow strip of four acres, lying to the west of the Boulder Clay area, separated from the latter by Lighter Drift soil and a hollow, down which flows a little stream and to which the land slopes sharply, contains soil of a Sedentary character. The latter is not very deep, but having a north-easterly aspect it is not very liable to suffer from drought.

Although the mechanical analysis of this soil (see Table III) shows it to contain a rather high proportion of clay and silt, yet the soil is of a light nature because it contains a high percentage of stones, especially so in the subsoil (see Table II).

Fringing this area on the western side is a narrow belt of wood, the latter being made up mainly of ash, oak and larch trees.

The distinction between the Sedentary soil and the Drift soils can be

easily recognised owing to the difference in both appearance and distribution of the stones.

(*b*) Arable Area.

Cae Gat Goch. The field has been continuously under the plough, apart from occasional rests in grass of from two to three years, and as a consequence contains an abnormally low amount of organic matter (see Table III, Sample 1).

It has a slight slope from the north side down to the south, but this does not amount to more than a few feet. It is of the Lighter Drift soil type, and it is only the road that separates this field from the Glacial Drift soil area of Cae'r Efail, which is of a similar origin and also resembles it in its mechanical composition (see Table III, Samples 13 and 1).

Thus the two fields afford excellent areas for comparison of the soil animal fauna. The difference in the constitution of the latter may be associated with cultural operations.

For the last two years the area was down under oats, and at the time of the investigation the land appeared in a clean condition.

4. Botanical Census of the Areas.

(*a*) Method of Investigation.

The method of procedure consisted in cutting turf samples of a standard size. Each turf sample measured six inches square and five random samples were taken within an area of 10 by 15 yards, near where the samples for the soil population had been taken in each of the pasture fields under consideration. In this way a representative sample was obtained of the botanical nature of each area examined for its soil animal fauna. Both the number of plant species and their relative abundance, per five samples of each area investigated, is given in Table I.

(*b*) Deductions from Botanical Analysis.

It will be noted that the Alluvial and Lighter Drift areas are good soil as indicated by the presence of *Alopecurus pratensis* in fairly large numbers, though in the Alluvial area the number of plants is probably lower than it might otherwise have been owing to conditions.

The Lighter Drift area is the best grazing agricultural sward, as it contains the least number of weeds, and the better grass species are present in fairly large numbers.

Table I.

Botanical Census (*dominant species only*).

	Cae'r Efail		Cae Mawr	
	Alluvial	Lighter Drift	Boulder Clay	Sedentary
Gramineae:				
Lolium perenne	—	85	95	85
Dactylis glomerata	—	34	53	27
Cynosurus cristatus	—	—	234	368
Alopecurus pratensis	57	388	8	4
Poa trivialis	430	413	460	225
Festuca rubra	53	106	—	10
Anthoxanthum odoratum	36	—	—	35
Leguminosae:				
*Trifolium repens**	10	4	109	190
Other orders (regarded as weeds):				
Holcus lanatus	160	213	347	390
Agrostis alba	28	55	149	103
Ranunculus repens	6	5	6	16
Juncus effusus	79	—	—	—
Geranium dissectum	22	—	10	8
Carex sp.	10	—	—	—
Veronica officinalis	20	—	4	14
Bellis perennis	—	—	8	37
Leontodon autumnale	—	—	2	14
Moss	Abundant	—	Common	Very common

* Of the 190 plants in Sedentary area, three were *Trifolium pratense.*

The presence of *Juncus* and *Carex* species in the Alluvial area indicates a marshy condition and the large number of weed species, *e.g. Geranium* and *Veronica* species, suggests a tendency for open spaces.

The absence of *Lolium perenne*, *Dactylis glomerata* and *Cynosurus cristatus* in the Alluvial area is a further indication of poor sward; their absence being due to the marshy nature of the soil.

The botanical analysis composition of the Boulder Clay and Sedentary indicates a very dense sward of "low plants." There are a large number of species of grasses accompanied by a high number of plants. These areas contain also large quantities of *Trifolium repens*, suggesting that these soils are rich in nitrogen.

5. Soil analysis of the areas examined.

(*a*) Mechanical Analysis.

Table II.

Stones and Coarse Gravel in various Types of Soil.

Type of soil	Ref. no. of sample	Soil: % stones above 1 cm. diam.	Soil: % coarse gravel 3 mm.–1 cm. diam.	Soil: Total	Subsoil: % stones above 1 cm. diam.	Subsoil: % coarse gravel 3 mm.–1 cm. diam.	Subsoil: Total
Alluvial	(3) Cae'r Efail	3·0	79·2	82·2	1·5	40·3	41·8
Lighter drift	(1) Gat Goch	29·3	30·9	60·2	112·0	66·0	178·0
Boulder clay	(11) Cae Mawr	10·4	40·0	50·4	0·5	50·0	50·5
Sedentary	(4) Bank	73·5	85·0	158·5	112·0	98·0	210·0

The above percentages have been calculated from the amount of air-dried soil passing through a 3 mm. sieve.

Table III.

Mechanical Analysis of Nantcellan Soils.

Type of soil	Alluvial	Lighter Drift		Boulder Clay	Sedentary
Name of field sample	Cae'r Efail (3)	Cae'r Efail (13)	Gat Goch (1)	Cae Mawr (17)	Cae Mawr (18)
Fine gravel ...	1·3	15·3	19·8	1·0	12·4
Coarse sand ...	1·3	11·4	9·5	2·2	7·6
Fine sand ...	3·9	10·1	11·0	12·8	16·9
Silt	12·5	15·3	11·5	14·4	12·2
Fine silt ...	33·3	29·0	26·9	35·7	24·0
Clay	21·5	7·1	9·1	15·4	11·0
Moisture ...	6·5	2·8	3·0	2·5	2·3
Loss on ignition	22·9	11·4	9·5	14·7	13·1
$CaCO_3$	Nil	0·01	0·02	—	—
		Subsoil.			
Fine gravel ...	0·0	20·9	33·2	0·0	14·4
Coarse sand ...	0·6	14·6	13·7	0·3	7·8
Fine sand ...	13·5	9·8	10·8	9·2	17·1
Silt	12·8	11·7	8·7	15·3	10·6
Fine silt ...	34·7	24·8	18·0	35·5	26·9
Clay	21·3	7·1	9·2	28·2	10·9
Moisture ...	3·7	2·3	2·8	1·6	1·7
Loss on ignition	11·1	8·5	6·1	9·8	8·9
$CaCO_3$	—	—	—	—	—

(*b*) Deductions from the Mechanical Analysis of the Soils.

It will be noted that the relative proportions of the total mineral ingredients of both the soil and subsoil of the two areas of the Glacial Lighter Drift soil formation are similar. These two areas are under similar environmental conditions, except that Cae'r Efail area is a permanent pasture and Gat Goch arable land, suggesting the possibility of expressing the difference in the soil fauna in terms of cultural operations.

There is in the Alluvial and Boulder Clay areas an exceptionally high percentage of fine silt accompanied by a high proportion of clay, which gives the soil its characteristic plastic nature. The amount of fine silt in the two areas is practically equal, but the total amount of clay is much greater in the Alluvial area. The percentage of fine gravel and coarse sand in both samples is very low, and does not increase in proportion on passing from soil to subsoil.

The relative proportion of clay and fine silt in the soil is important, not only in that it hinders the rapid movement of soil inhabitants, but that it also determines the amount of gravitational water, which is very often the detrimental factor of soil life. The finer the soil particles the greater the pore space, and consequently the greater the quantity of water retained. Also, the slower the percolation of water through such soil, the greater the capillary capacity will be; thus the soil becomes waterlogged after heavy rainfalls. Under the latter conditions all soil air is expelled and the water therein will contain less dissolved air or oxygen and the rapid diffusion of soil air in all directions will be lessened owing to lowering of the temperature. So both the animals and the plants will suffer, the former due to the absence of free air, the latter to deficiency of dissolved air.

The mechanical analysis composition of the Heavier Drift soil indicates a Boulder Clay formation, but the high amount of organic matter, the natural slope of the land facilitating drainage, makes it more or less of the nature of a lighter type of soil.

The Alluvial soil has a mineral composition similar to that of the Boulder Clay soil, but has a much higher percentage of organic matter. Owing to the low situation of the field, and the absence of sufficient drainage, the high amount of organic matter by no means obliterates the harmful effect of the fine silt and clay on the texture of the soil. It would thus appear that the difference in the fauna of these two soil types may be due mainly to the high water content of the Alluvial soil.

It will be further noted that the Lighter Drift soil contains a much

higher percentage of fine gravel and coarse sand than the Boulder Clay soil; thus an attempt may be made to relate faunistic differences to a difference in size of soil particles.

For an examination of the mechanical analysis of the fine earth of the sedentary soil (Table III), one would regard it as of a heavy nature, but as it contains such a high proportion of stones (Table II, sample 4) and rather a high percentage of organic matter (Table III, sample 18), its heavy nature is modified to such an extent that it has some of the characteristics of a light soil. This area resembles in its mechanical formation that of the Lighter Drift soil area of Cae'r Efail, thus suggesting an attempt to relate faunistic differences to differences in stone content, the latter being in turn related to geological differences.

(*c*) Deductions from the Chemical Analysis of the Soils.

Chemical analyses were carried out and it was found that there is a remarkable similarity in the total amounts of mineral ingredients present in different types of soil. As a consequence, the possibility that any of the essential mineral ingredients necessary for the natural growth of plants might be a determining factor of importance in relation to faunal differences between the investigated areas is reduced to a minimum.

The amount of lime present is negligible but the reactions of the majority of the soils indicate that no marked sourness has developed and no bad effects on the herbage could be traced. Undoubtedly, more liming would have an appreciable effect on the fertility of the Boulder Clay, as it would ameliorate the unfavourable physical properties caused by the high proportion of clay.

6. METHOD OF INVESTIGATION OF FAUNA.

The method of taking the sample was in the main the same as that employed by Morris, except that the lower six inches was taken, in two samples of three inches each. The deeper samples are thus comparable with those of Thompson (1924) rather than those of Morris (1922) who divided his lower six inches into three samples of two inches each.

The soil was removed in this way to a depth of nine inches, giving four samples, which consisted of: I, the soil between the surface and a depth of one inch; II, the soil between a depth of one inch and a depth of three inches; III, the soil between three inches and six inches; IV, the soil between six inches and nine inches. The samples thus obtained were taken to the laboratory and examined. By taking a small quantity of

soil at a time, and examining it carefully, it was possible to obtain most of the insects and their larvae, Nematoda, Annelida and Mollusca, except Coleopterous adults feigning death, a few larvae of Coleoptera and Diptera and most Collembola and Acarina.

After this preliminary sifting, a portion at a time of the soil was taken and washed through a series of three sieves each eighteen inches in diameter, with meshes of 3·5 mm., 1·5 mm. and fifty meshes to the linear inch, respectively. The mesh of final sieve was the same as that used by Thompson, and was small enough to retain all the insects and their larvae, and the Acarina that were not detected by the preliminary sorting. The sieves at the same time were shaken to allow the finest soil particles to pass through the meshes. When all the sediment had passed through, both residue and filtrate were examined. No organisms were ever found in the filtrate, so after the preliminary experiments the filtrate was neglected.

The residue on each sieve was then washed out separately into a shallow dish, and water added until the dish was half full. As the clay had all been washed out, the water remained clear, and by gently stirring the residue the organisms between the soil particles were released, and either floated on the surface of the water or remained on the surface of the residue at the bottom of the dish. The process was repeated until nothing further was found; usually one stirring was sufficient in the case of the residue retained by the uppermost two sieves. Finally the actual solid residue was examined but very rarely was anything present except a few small Oligochaeta and Nematoda.

In most cases, the larvae and pupae of insects could not be specifically identified and it was only by breeding out of the adult forms that they could be determined with exactness. At the same time, when plenty of material was at hand, some of them were preserved in 4 per cent. formalin or mounted on slides. All the adults reared were either set or preserved in formalin or 75 per cent. alcohol, along with, in most cases, one or more larvae and pupae of their particular species. When success did not attend the rearing of a species, it was nevertheless possible in most cases to indicate its family and sometimes also its genus.

The investigation is intended more to illustrate the differences between the different types of soil than seasonal variation.

Four samples were examined from each of the five soil types and were taken in January 1925, July, October, and January 1926 respectively. The samples of the different soils taken in any given month were taken at dates as close together as possible. Care was taken in selecting the

samples to avoid patches influenced by any special factors, such as proximity of hedges, etc.

I express my thanks for help in the identification of species of Insecta, Myriapoda and Mollusca to Messrs S. G. Brade-Birks, G. C. Robson, G. H. Carpenter, F. W. Edwards, R. Stenton, and B. S. Williams.

7. FAUNAL CENSUS OF INVESTIGATED AREAS.

In the following list the Oligochaeta have been divided into two groups: (*a*) Terricolae, which includes such forms as *Lumbricus terrestris*, and (*b*) Limicolae, which includes the small white forms. The formula used is based on that used by Morris (1922). The numbers outside the brackets give the number of the sample[1] (or samples) in which the species occurred and the letters the areas in which they were found, A. = Alluvial pasture, B. = Lighter Drift pasture, C. = Lighter Drift arable, D. = Boulder Clay pasture, E. = Sedentary pasture. The first numbers within the brackets give, above, the total number found, and below, in Roman numerals, the layer (or layers) in which they were found. The second numbers within brackets give, above, the greatest number found at any one level, and below, in Roman numerals, the level at which they were found. The letter (or letters) in brackets placed after the name of a species indicate the stage of development, *i.e.* (L.)—Larval; (P.)—Pupal; (A.)—Adult.

Species common in all Areas.

* Indicates that insects have been reared by the writer from larvae found in species thus marked.

Nematoda. Spp. A. 1, 3 $\left(\frac{6}{\text{I, II}}; \frac{4}{\text{I}}\right)$; B. 1, 3, 4 $\left(\frac{45}{\text{I–IV}}; \frac{18}{\text{III}}\right)$; C. 1, 3, 4 $\left(\frac{28}{\text{I–IV}}; \frac{9}{\text{III}}\right)$; D. 1, 3, 4 $\left(\frac{8}{\text{II–IV}}; \frac{3}{\text{IV}}\right)$; E. 1–4 $\left(\frac{66}{\text{I–IV}}; \frac{13}{\text{IV}}\right)$.

Oligochaeta. (Terricolae.) A. 1-4 $\left(\frac{94}{\text{I–III}}; \frac{48}{\text{I}}\right)$; B. 1-4 $\left(\frac{78}{\text{I-IV}}; \frac{19}{\text{I}}\right)$; C. 1-4 $\left(\frac{31}{\text{I–IV}}; \frac{6}{\text{IV}}\right)$; D. 1-4 $\left(\frac{116}{\text{I–IV}}; \frac{33}{\text{I}}\right)$; E. 1–4 $\left(\frac{89}{\text{I–IV}}; \frac{19}{\text{I}}\right)$.

(Terricolae.) Cocoons. A. 1, 3, 4 $\left(\frac{36}{\text{I, II}}; \frac{18}{\text{I}}\right)$; B. 1-4 $\left(\frac{63}{\text{I-IV}}; \frac{14}{\text{II}}\right)$; C. 1, 3, 4 $\left(\frac{26}{\text{II–IV}}; \frac{4}{\text{II}}\right)$; D. 1–4 $\left(\frac{112}{\text{I-IV}}; \frac{65}{\text{I}}\right)$; E. 1–4 $\left(\frac{130}{\text{I–IV}}; \frac{58}{\text{I}}\right)$.

(Limicolae.) A. 1-4 $\left(\frac{344}{\text{I, II}}; \frac{135}{\text{I}}\right)$; B. 1–4 $\left(\frac{292}{\text{I–IV}}; \frac{77}{\text{I}}\right)$; C. 1-4 $\left(\frac{83}{\text{II–IV}}; \frac{6}{\text{III}}\right)$; D. 1–4 $\left(\frac{385}{\text{I–IV}}; \frac{106}{\text{I}}\right)$; E. 1–4 $\left(\frac{355}{\text{I–IV}}; \frac{72}{\text{I}}\right)$.

[1] *I.e.* 1 = sample taken in January 1925; 2 = in July; 3 = in October; 4 = in January 1926.

ARACHNIDA. Araneida. Spp. A. 3, 4 $\left(\frac{3}{I}\right)$; B. 2, 3 $\left(\frac{5}{I}\right)$; C. 3, 4 $\left(\frac{2}{I}\right)$; D. 2-4 $\left(\frac{6}{I}\right)$; E. 2–4 $\left(\frac{6}{I}\right)$.

Acarina. Spp. A. 1–4 $\left(\frac{228}{I, II}; \frac{102}{I}\right)$; B. 1–4 $\left(\frac{178}{I, II, IV}; \frac{60}{I}\right)$; C. 1–4 $\left(\frac{59}{I–III}; \frac{13}{I}\right)$; D. 1–4 $\left(\frac{95}{I–IV}; \frac{46}{I}\right)$; E. 1–4 $\left(\frac{128}{I, II}; \frac{50}{I}\right)$.

INSECTA. Collembola. *Onychiurus armatus* (Tulb.) A. 2–4 $\left(\frac{30}{I–IV}; \frac{12}{III}\right)$; B. 1–4 $\left(\frac{899}{I–IV}; \frac{160}{I}\right)$; C. 1–4 $\left(\frac{540}{I–IV}; \frac{77}{II}\right)$; D. 1–4 $\left(\frac{85}{I–IV}; \frac{46}{I}\right)$; E. 1–4 $\left(\frac{42}{I–IV}; \frac{12}{I}\right)$; *Achorutes armatus* (Nic.) A. 4 $\left(\frac{4}{I}\right)$; B. 1–4 $\left(\frac{87}{I–IV}; \frac{21}{I}\right)$; C. 4 $\left(\frac{41}{I–IV}; \frac{15}{I}\right)$; D. 1–4 $\left(\frac{130}{I, II}; \frac{51}{I}\right)$; E. 1–4 $\left(\frac{125}{I–IV}; \frac{60}{I}\right)$; *Isotoma viridis* Bourl. A. 2–4 $\left(\frac{43}{I}; \frac{25}{I}\right)$; B. 1–4 $\left(\frac{40}{I, II}; \frac{15}{I}\right)$; C. 1, 3 $\left(\frac{7}{I, II}\right)$; D. 1–4 $\left(\frac{20}{I, II}; \frac{8}{I}\right)$; E. 1–4 $\left(\frac{40}{I, II}; \frac{21}{I}\right)$; *Isotomurus palustris* (Müll.) A. 3–4 $\left(\frac{22}{I–III}; \frac{13}{I}\right)$; B. 3, 4 $\left(\frac{2}{I, IV}\right)$; C. 3 $\left(\frac{1}{I}\right)$; D. 1–4 $\left(\frac{55}{I–IV}; \frac{19}{I}\right)$; E. 1, 3 $\left(\frac{13}{I–III}; \frac{7}{I}\right)$.

Immature forms of the following families also occurred:

Onychiuridae. A. 4 $\left(\frac{4}{III}\right)$; B. 2, 3 $\left(\frac{5}{I–III}\right)$; C. 3 $\left(\frac{21}{I}\right)$; D. 2, 4 $\left(\frac{24}{I–IV}; \frac{12}{I}\right)$; E. 2 $\left(\frac{4}{III, IV}\right)$; Isotomidae. A. 3, 4 $\left(\frac{17}{I}; \frac{10}{I}\right)$; B. 3, 4 $\left(\frac{7}{I}; \frac{4}{I}\right)$; C. 1–4 $\left(\frac{7}{I, II}; \frac{2}{I}\right)$; D. 2, 3 $\left(\frac{5}{I}; \frac{3}{I}\right)$; E. 3 $\left(\frac{1}{I}\right)$.

Coleoptera. *Atheta analis* A. (A). A. 3, 4 $\left(\frac{3}{I}\right)$; B. 2, 3 $\left(\frac{3}{I, II}\right)$; C. 3 $\left(\frac{1}{I}\right)$; D. 1, 2, 4 $\left(\frac{3}{I}\right)$; E. 3, 4 $\left(\frac{3}{I}\right)$.

Diptera. *Limosina sylvatica* Mg.* A. 3, 4 $\left(\frac{2}{I}\right)$; B. 3 $\left(\frac{3}{I}\right)$; C. 1, 4 $\left(\frac{9}{II, III}; \frac{4}{III}\right)$; D. 1, 3, 4 $\left(\frac{9}{I, II}; \frac{4}{I}\right)$; E. 1, 3, 4 $\left(\frac{7}{I}\right)$.

Species occurring in from two to four Areas.

GASTROPODA. *Hyalinia radiatula* Alder. A. 1, 3, 4 $\left(\frac{6}{I}; \frac{2}{I}\right)$; B. 1, 4 $\left(\frac{2}{I}\right)$; E. 3 $\left(\frac{1}{I}\right)$; *Vallonia pulchella* Müll. B. 1, 3, 4 $\left(\frac{10}{I}; \frac{5}{I}\right)$; E. 2–4 $\left(\frac{7}{I}; \frac{3}{I}\right)$; *Cochlicopa lubrica* Müll. A. 1, 3, 4 $\left(\frac{9}{I}; \frac{4}{I}\right)$; E. 3, 4 $\left(\frac{2}{I}\right)$; *Carychium minimum* Müll. A. 4 $\left(\frac{1}{I}\right)$; D. 1, 4 $\left(\frac{2}{I}\right)$; *Limax agrestis* (Linne) D. 3 $\left(\frac{2}{I}\right)$; E. 1 $\left(\frac{1}{I}\right)$; *Limax* spp. B. 1 $\left(\frac{3}{I}\right)$; C. 1, 3 $\left(\frac{2}{I}\right)$; D. 4 $\left(\frac{3}{I}\right)$; E. 3, 4 $\left(\frac{3}{I}\right)$.

MYRIAPODA. *Brachyiulus* (*Microbrachyiulus*) *pusillus* (Leach) C. 1–4 $\left(\frac{34}{I–IV}; \frac{4}{I}\right)$; D. 3 $\left(\frac{I}{III}\right)$; *Geophilus longicornis* Leach B. 1 $\left(\frac{3}{II, IV}\right)$; C. 1–4 $\left(\frac{14}{I–IV}; \frac{3}{III}\right)$; D. 1 $\left(\frac{1}{I}\right)$;

E. 4 $\left(\frac{3}{\text{I, III}}; \frac{2}{\text{I}}\right)$; Symphyla spp. B. 1–4 $\left(\frac{58}{\text{I–IV}}; \frac{18}{\text{IV}}\right)$; C. 1–4 $\left(\frac{162}{\text{I–IV}}; \frac{61}{\text{IV}}\right)$; D. 1-4 $\left(\frac{39}{\text{III, IV}}; \frac{17}{\text{IV}}\right)$; E. 1–4 $\left(\frac{55}{\text{II–IV}}; \frac{21}{\text{IV}}\right)$.

INSECTA. Collembola. *Onychiurus fimetarius* (Linn.) A. 3 $\left(\frac{5}{\text{I}}\right)$; B. 1, 3 $\left(\frac{10}{\text{I, II}}; \frac{6}{\text{I}}\right)$; C. 3 $\left(\frac{2}{\text{I}}\right)$; *Tullbergia quadrispina* (Börn.) B. 1–4 $\left(\frac{224}{\text{II–IV}}; \frac{41}{\text{III}}\right)$; C. 1–4 $\left(\frac{998}{\text{I–IV}}; \frac{150}{\text{III}}\right)$; D. 1–4 $\left(\frac{394}{\text{I–IV}}; \frac{90}{\text{I}}\right)$; E. 1–4 $\left(\frac{584}{\text{I–IV}}; \frac{90}{\text{IV}}\right)$; *Folsomia fimetaria* (Linn.) B. 1–4 $\left(\frac{143}{\text{I–IV}}; \frac{41}{\text{II}}\right)$; C. 1–4 $\left(\frac{30}{\text{II, III}}; \frac{10}{\text{II}}\right)$; E. 1–4 $\left(\frac{95}{\text{I–IV}}; \frac{35}{\text{I}}\right)$; *Sminthurus viridis* Lubb. B. 2 $\left(\frac{1}{\text{I}}\right)$; C. 3 $\left(\frac{5}{\text{I–III}}; \frac{2}{\text{I}}\right)$; D. 1 $\left(\frac{1}{\text{IV}}\right)$.

Thysanoptera. Spp. B. 3 $\left(\frac{1}{\text{I}}\right)$; C. 3, 4 $\left(\frac{3}{\text{I–III}}\right)$.

Rhynchota. *Aphis* spp. A. 3 $\left(\frac{1}{\text{I}}\right)$; B. 3 $\left(\frac{6}{\text{I}}\right)$; D. 3, 4 $\left(\frac{6}{\text{I}}\right)$; E. 2, 3 $\left(\frac{7}{\text{I}}\right)$.

Lepidoptera. Spp. B. 3 $\left(\frac{1}{\text{II}}\right)$; C. 1 $\left(\frac{1}{\text{I}}\right)$; D. 3 $\left(\frac{1}{\text{I}}\right)$; E. 1, 4 $\left(\frac{3}{\text{I}}\right)$.

Coleoptera. *Xantholinus longiventris* Heer. (A.) B. 4 $\left(\frac{1}{\text{I}}\right)$; C. 1, 4 $\left(\frac{2}{\text{I}}\right)$; D. 3 $\left(\frac{1}{\text{I}}\right)$; E. 3 $\left(\frac{2}{\text{I}}\right)$; *Philonthus nigritulus* Grav. (A.) A. 4 $\left(\frac{2}{\text{I}}\right)$; D. 4 $\left(\frac{1}{\text{I}}\right)$; E. 1 $\left(\frac{1}{\text{I}}\right)$; *Stenus brunnipes* Steph. (A.) B. 3 $\left(\frac{1}{\text{I}}\right)$; D. 1 $\left(\frac{1}{\text{I}}\right)$; *Tachyporus brunneus* Fab. (A.) B. 4 $\left(\frac{1}{\text{I}}\right)$; D. 1 $\left(\frac{1}{\text{I}}\right)$; E. 3 $\left(\frac{1}{\text{I}}\right)$; *Trichopteryx* spp. (A.) B. 1, 3 $\left(\frac{2}{\text{I}}\right)$; E. 1, 3 $\left(\frac{3}{\text{I}}\right)$; *Canthuris* (*Telophorus*) *rufa* Linn. var. *lituratus* Fall.* (L.) A. 4 $\left(\frac{6}{\text{I}}\right)$; B. 4 $\left(\frac{4}{\text{I}}\right)$; E. 3 $\left(\frac{1}{\text{I}}\right)$; *Dryops* (*Parnus*) sp. (A.) A. 2 $\left(\frac{1}{\text{I}}\right)$; B. 2, 4 $\left(\frac{2}{\text{I}}\right)$; *Agriotes obscuris* L. (L.) A. 3 $\left(\frac{1}{\text{II}}\right)$; B. 1–4 $\left(\frac{3}{\text{I, II}}\right)$; D. 3, 4 $\left(\frac{5}{\text{I}}\right)$; E. 3 $\left(\frac{1}{\text{II}}\right)$; *Athous haemorrhoidalis* F. (L.) A. 3 $\left(\frac{1}{\text{II}}\right)$; D. 1–4 $\left(\frac{8}{\text{I–IV}}; \frac{2}{\text{III}}\right)$. Unidentified larvae and pupae: STAPHYLINIDAE B. 4 $\left(\frac{1}{\text{I}}\right)$; C. 1–4 $\left(\frac{4}{\text{I–III}}\right)$; D. 2 $\left(\frac{5}{\text{I, IV}}; \frac{2}{\text{I}}\right)$; E. 3, 4 $\left(\frac{6}{\text{I, III}}; \frac{2}{\text{I}}\right)$; unclassified B. 2, 4 $\left(\frac{18}{\text{I, II}}; \frac{8}{\text{IV}}\right)$; C. 3, 4 $\left(\frac{2}{\text{I}}\right)$; D. 3 $\left(\frac{1}{\text{I}}\right)$; E. 2, 3 $\left(\frac{2}{\text{I}}\right)$.

Diptera. *Oligotrophus alopecuri* Reut. (?)* A. 1, 3, 4 $\left(\frac{245}{\text{I, II}}; \frac{235}{\text{I}}\right)$; E. 4 $\left(\frac{1}{\text{I}}\right)$; *Sciara annulata* Mg.* B. 3, 4 $\left(\frac{2}{\text{I}}\right)$; D. 1–3 $\left(\frac{8}{\text{I}}\right)$; *Bibio johannis* L. (L.) A. 3 $\left(\frac{8}{\text{I}}\right)$; B. 4 $\left(\frac{10}{\text{I}}\right)$; C. 1, 4 $\left(\frac{7}{\text{I, III}}; \frac{6}{\text{III}}\right)$; E. 3, 4 $\left(\frac{36}{\text{I}}\right)$; *Tipula oleracea* L.* B. 3 $\left(\frac{1}{\text{I}}\right)$; D. 1 $\left(\frac{2}{\text{I}}\right)$; E. 1 $\left(\frac{1}{\text{I}}\right)$; T. spp. (L.) B. 2, 4 $\left(\frac{4}{\text{I}}\right)$; C. 4 $\left(\frac{2}{\text{I}}\right)$; E. 3, 4 $\left(\frac{3}{\text{I}}\right)$; *Empis livida** A. 1–4 $\left(\frac{9}{\text{I}}\right)$; B. 3–4 $\left(\frac{5}{\text{I}}\right)$; E $\left(\frac{1}{\text{I}}\right)$; *Ramphomyia spinipes* Fln.* B. 1, 3 $\left(\frac{12}{\text{I–III}}; \frac{8}{\text{II}}\right)$; D. 2, 4 $\left(\frac{3}{\text{I, II}}; \frac{2}{\text{I}}\right)$; E. 1, 3, 4

$\left(\frac{7}{\text{I–IV}}; \frac{5}{\text{I}}\right)$; *Chloromyia formosa* Scop.* D. 3, 4 $\left(\frac{13}{\text{I}}\right)$; E. 4 $\left(\frac{1}{\text{I}}\right)$; *Coelopa pilipes* Hal.* B. 1, 3 $\left(\frac{12}{\text{I–III}}; \frac{7}{\text{II}}\right)$; C. 1 $\left(\frac{1}{\text{III}}\right)$; D. 2, 4 $\left(\frac{2}{\text{I, II}}\right)$; E. 1, 3, 4 $\left(\frac{7}{\text{I–IV}}; \frac{4}{\text{I}}\right)$; *Polietes albineata* F. C. 1 $\left(\frac{6}{\text{II, III}}; \frac{5}{\text{III}}\right)$; E. 4 $\left(\frac{1}{\text{I}}\right)$; *Tanypus maculatus* (L.) A. 1 $\left(\frac{1}{\text{I}}\right)$; C. 1 $\left(\frac{1}{\text{I}}\right)$; D. 1, 2 $\left(\frac{3}{\text{I, II}}\right)$; E. 1 $\left(\frac{1}{\text{I}}\right)$; *Helobia* sp. (L.) A. 1, 4 $\left(\frac{33}{\text{I, II}}; \frac{24}{\text{I}}\right)$; E. 1 $\left(\frac{1}{\text{I}}\right)$. Unidentified larvae and pupa: CECIDOMYIDAE B. 3 $\left(\frac{2}{\text{I}}\right)$; C. 3 $\left(\frac{1}{\text{I}}\right)$; D. 1–4 $\left(\frac{28}{\text{I}}\right)$; E. 3 $\left(\frac{9}{\text{I}}\right)$; MYCETOPHILIDAE D. 4 $\left(\frac{72}{\text{I–III}}; \frac{45}{\text{III}}\right)$; E. 4 $\left(\frac{31}{\text{I}}\right)$; unclassified (L.) A. 4 $\left(\frac{1}{\text{I}}\right)$; B. 4 $\left(\frac{15}{\text{I–III}}; \frac{8}{\text{I}}\right)$; D. 2, 3, 4 $\left(\frac{18}{\text{I}}\right)$; E. 3, 4 $\left(\frac{9}{\text{I}}\right)$.

Species confined to a single Area.

MYRIAPODA. *Blaniulus guttulatus* (Bosc.) C. 1–4 $\left(\frac{15}{\text{I–III}}; \frac{4}{\text{I}}\right)$; *Monotarsobius duboscqui* (Brolemann) C. 1–4 $\left(\frac{13}{\text{I–IV}}; \frac{2}{\text{I}}\right)$; *Geophilus insculptus* Attems. C. 3 $\left(\frac{2}{\text{I, IV}}\right)$; *Schendyla memorensis* (C. L. Koch) C. 1–4 $\left(\frac{4}{\text{II–IV}}\right)$; *Brachydesmus superus moscellanus* Verhoeff. C. 3 $\left(\frac{3}{\text{I, II}}; \frac{2}{\text{I}}\right)$.

INSECTA. Collembola. *Schottella parvula* (Schäff.) C. 3 $\left(\frac{14}{\text{I}}\right)$; *Xenylla brevicauda* Tulb. B. 3 $\left(\frac{1}{\text{IV}}\right)$; *Folsomia quadrivculata* (Tulb.) A. 1–4 $\left(\frac{130}{\text{I–IV}}; \frac{57}{\text{I}}\right)$; *Sinella curviseta* Brook. D. 2 $\left(\frac{8}{\text{III, IV}}\right)$; *Sminthurus aureus* (Lubb.) B. 1, 3 $\left(\frac{2}{\text{I, III}}\right)$; *Bourletiella lutea* (Lubb.) C. 4 $\left(\frac{7}{\text{II}}\right)$; *Arrhopalites coecus* (Tulb.) B. 4 $\left(\frac{1}{\text{II}}\right)$.

Thysanura. Sp. E. 2 $\left(\frac{1}{\text{I}}\right)$.

Rhynchota. *Athysanus communis* J. Sahl. B. 3 $\left(\frac{1}{\text{I}}\right)$.

Coleoptera. *Nebria brevicollis* F. (A.) C. 3 $\left(\frac{1}{\text{I}}\right)$; *Pterostichus vernalis* Gyll. (A.) E. 4 $\left(\frac{1}{\text{I}}\right)$; *Notiophilus substriatus* Wat. (A.) C. 3 $\left(\frac{1}{\text{I}}\right)$; *Bembidium obtusum* Sturm. (A.) D. 2 $\left(\frac{1}{\text{I}}\right)$; CARABIDAE sp. (L.) E. 1 $\left(\frac{1}{\text{I}}\right)$; *Xantholinus linearis* Ol. (A.) E. 4 $\left(\frac{2}{\text{I}}\right)$; *X. punctulatus* (Payk.) (A.) B. 1 $\left(\frac{1}{\text{I}}\right)$; *Quedius boops* Grav. (A.) D. 3 $\left(\frac{1}{\text{I}}\right)$; *Othius laeviusculus* Steph. (A.) D. 3 $\left(\frac{1}{\text{I}}\right)$; *Philonthus politus* F. (A.) E. 3 $\left(\frac{1}{\text{I}}\right)$; *P. umbratilis* Grav. (A.) D. 1 $\left(\frac{1}{\text{I}}\right)$; *P. varius* Gyll. (A.) E. 1 $\left(\frac{1}{\text{I}}\right)$; *Platystethus arenarius* Fourc. (A.) B. 4 $\left(\frac{1}{\text{I}}\right)$; *Oxytelus tetracarinatus* (Block) (A.) A. 2 $\left(\frac{1}{\text{IV}}\right)$; *O. sculpturatus* Grav. (A.) D. 4 $\left(\frac{1}{\text{I}}\right)$; *Stenus declaratus* Er. (A.)

E. 1 $\left(\frac{1}{I}\right)$; *Tachyporus obtusus* L. (A.) B. 2 $\left(\frac{1}{I}\right)$; *Mycetoporus splendidulus* (A.) D. 3 $\left(\frac{1}{I}\right)$; *Tachinus laticollis* Grav. (A.) C. 2 $\left(\frac{1}{I}\right)$; *Helophorus aeneipennis* Thoms. (A.) A. 3 $\left(\frac{1}{I}\right)$; *Cercyon melanocephalus* L. (A.) E. 3 $\left(\frac{1}{I}\right)$; *C. lateralis* Marsh. (A.) D. 3 $\left(\frac{1}{I}\right)$; *Megasternum boletophagum* Marsh. (A.) D. 4 $\left(\frac{1}{I}\right)$; *Cartodere filum* Aube. (A.) E. 2 $\left(\frac{1}{IV}\right)$; *Ptinus tectus* F. (A.) B. 1 $\left(\frac{1}{IV}\right)$. *Aphodius fimetarius* L. (A.) B. 3 $\left(\frac{1}{I}\right)$; *A. punctatosulcatus* Sturm. (A.) E. 1 $\left(\frac{1}{I}\right)$; *Agriotes sputator* (?) (L.) D. 4 $\left(\frac{1}{II}\right)$; *Sitones lineatus* (A.) C. 1–3 $\left(\frac{3}{I}\right)$; *Liosoma ovatulum* Clairv. (A.) D. 4 $\left(\frac{1}{I}\right)$; *Barynotus obscuris* (A.) D. 1 $\left(\frac{1}{I}\right)$.

Hymenoptera. *Cynipidae* sp. (A.) B. 3 $\left(\frac{1}{I}\right)$; *Proctotrypidae* sp. (A.) D. 2, 3 $\left(\frac{2}{I}\right)$; *Pelecinidae* sp. (A.) 3 $\left(\frac{1}{I}\right)$; *Braconidae* sp. (A.) E. 3 $\left(\frac{1}{III}\right)$; *Myrmica scabrinodes* (A.P.L.) E. 1–3 $\left(\frac{463}{I–IV}; \frac{263}{II}\right)$.

Diptera. *Phronia* (?) *perdita* Mg.* (P.) E. 1 $\left(\frac{1}{I}\right)$; *Dilophus febrilis* L.* E. 1 $\left(\frac{3}{I}\right)$; *Bibio venosus* Mg.* C. 1 $\left(\frac{13}{III}\right)$; *Leptis scolopacea* L.* B. 1 $\left(\frac{5}{I–III}; \frac{3}{I}\right)$; *Tipula ochracea* Mg.* D. 1 $\left(\frac{1}{I}\right)$; *T. lateralis* Mg.* C. 1 $\left(\frac{3}{I}\right)$; *Rhyphus punctatus* F.* E. 1 $\left(\frac{2}{I}\right)$; *Scatophago stercoraria* L.* D. 2, 3 $\left(\frac{2}{I}\right)$; *Borborus geniculatus* Mcq. E. 2 $\left(\frac{1}{I}\right)$; *Anthomyia* sp.* D. 3 $\left(\frac{2}{I}\right)$; *Ceratopogen* sp. (L.) A. 1, 3, 4 $\left(\frac{22}{I}\right)$; *Camptocladius* sp. (L.) A. 3 $\left(\frac{94}{I–IV}; \frac{40}{I}\right)$; *Gnophomyia* sp. (L.) A. 1 $\left(\frac{1}{I}\right)$; *Hexatominae* sp. (L.) 1 $\left(\frac{1}{I}\right)$; *Aphiochaeta brevicosalis* Wood* B. 2 $\left(\frac{37}{I}\right)$.

8. DISCUSSION OF DATA FURNISHED BY FAUNAL CENSUS.

A. Total Fauna.

(*a*) *In Relation to the Different Soil Types.* The total number of invertebrates per sample is much higher than has been indicated by previous workers on soil animal ecology, except in the case of Thompson (1924), who also worked on Aberystwyth soil. This is due, probably, to the further refinement of methods adopted by Thompson and in the present investigation for separating and detecting the soil organisms, especially the Acarina and Collembola.

There is considerable variation in the total number of invertebrates found in the different soil types (Figs. 1 and 2). The Alluvial contained the lowest number and it is suggested that the differences between its numbers and those of the Boulder Clay pasture may be due to the high water content of the former. If this be so it follows that the situation of

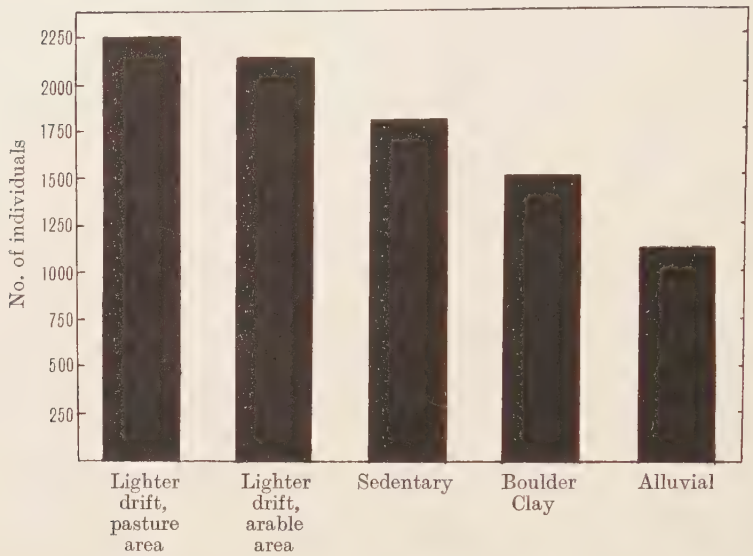

Fig. 1. Relative frequency of occurrence of soil fauna in the five areas.

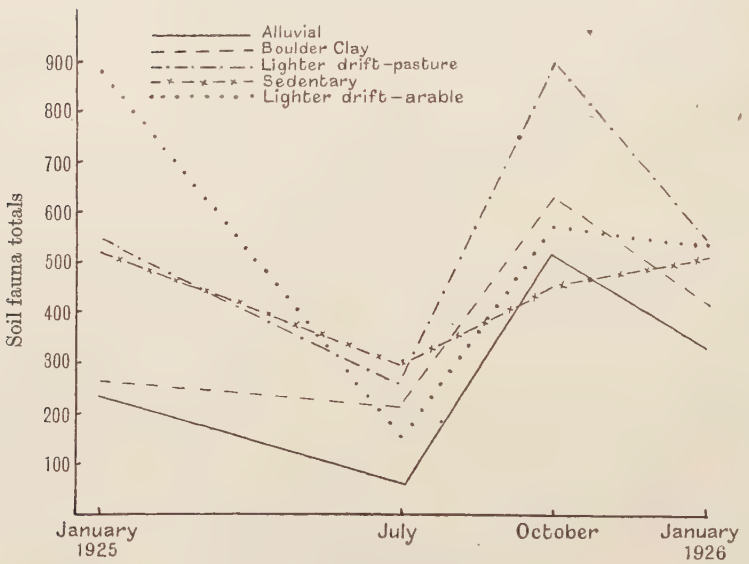

Fig. 2. Total fauna curves.

the land may have a considerable effect on the fauna in that it affects the amount of gravitational water present. Ground water is most destructive to soil inhabitants, not only because the spaces between the soil particles become completely filled with water, thus expelling the free soil air, but the organisms are much more liable to fungoid attacks. It will be noticed that the Lighter Drift soil differs from the Boulder Clay soil mainly in the larger size of soil particles (Tables II and III), and it is therefore probable that the quantitative faunistic difference between the two may be due to a difference in their water-holding power. The latter increases with the decrease in size of the soil particles, and the atmosphere available to subterranean animals differs accordingly in different soils. The degree of aeration of a soil is of equal importance to the fauna indirectly as oxygen is essential for most actions and interactions of the factors operating in the soil.

Ants occurred in three samples examined from the Sedentary area, but have not been included in the totals from which the figures were drawn.

It is seen that there was a marked difference in the total numbers of invertebrates obtained from the Sedentary and Lighter Drift pastures respectively, as illustrated in Fig. 1. This difference was largely due to the lower number found in the sample examined in October from the Sedentary area (Fig. 2). Omitting the October difference, the numbers found were remarkably similar in the two soils. It may be suggested that the difference in October should be associated with the nature of the flora. The predominant grass in the Lighter Drift is *Alopecurus pratensis*, which is a tall and very leafy plant compared with *Cynosurus cristatus*, which is the most abundant grass in the Sedentary area (Table I). The former grass offers the greater shelter and protection, especially in autumn, and is the more efficient in the prevention of undue evaporation of moisture from the soil.

Contrary to the experience of previous workers, cultural operations did not seem to have an appreciable detrimental effect on the total population in the Arable area, as compared with the Lighter Drift pasture. This was due to the higher numbers of Collembola and Myriapoda found in most of the samples from the Arable area. Analysis shows, however, that it is the Collembola, Onychiuridae spp. which are of importance in Lighter Drift arable and Lighter Drift pasture respectively. The relatively high position of the arable curve in January 1925 is due to the high proportion of *Tullbergia quadrispina* present, whereas the high position of the pasture curve in October is influenced by an increase in number of *Onychiurus armatus*.

Fig. 2 shows the quantitative changes in the total fauna for each of the soil types at different times of the year. The great difference in the levels of the curves in July and October might be explained as being related, not only to the normal seasonal variation at these times of the year, but to the unusual dryness of seven weeks previous to the taking of samples in July. This conclusion is further confirmed by the fact that shrivelled dead bodies of Collembola and Symphyla were found in considerable quantities.

The total number found in samples taken in January 1925 and the corresponding time in the following year was almost identical in the case of the Sedentary and Lighter Drift pastures (Fig. 2). In the late spring and early summer 1925, while the investigation was in progress, the drainage of both the Alluvial and the Boulder Clay areas was greatly improved. This led to a remarkable improvement in the conditions of these areas. Appreciable increase is observed in the total fauna of the samples taken in January 1925, as compared with the ones made at the corresponding time in the previous year (Fig. 2). This improvement was not confined to the uppermost layer, but extended down to the third layer.

All the soils under consideration are rich in organic matter, and even the subsoil seems to contain a fair amount, as indicated in Table III (under heading "Loss on ignition"). Though the arable area contains the least amount, yet, excepting the Lighter Drift pasture, this area has the highest amount of organisms. Thus it follows, firstly, that the differences in the amount of organic matter are not the effective factor in determining the total soil population in the pasture areas examined, and secondly, seeing that even the subsoils of the investigated grasslands are nearly as rich in organic matter as the soil of the arable area, it is highly probable that the organic content is not the determining factor in the vertical distribution.

(*b*) *In Relation to Depth.* The depth at which soil organisms occurred in the five areas investigated is of considerable interest (Fig. 3). That the fauna of the Alluvial area was almost exclusively confined to the uppermost inch layer may probably be associated with the high water level, owing to its low-lying situation. This conclusion is further borne out by the fact that the improved drainage of the ground water into lower levels, made during summer 1925, was followed by a partial restoration of the fauna in the lower levels.

The fauna of the Boulder Clay area, though largely found in the surface inch layer, was more evenly distributed and more abundant in the lower layers than that of the Alluvial soil. Contrary to expectation, the

third layer contained a higher number of individuals than the second layer (Fig. 3). This observation suggested the advisability of making a mechanical analysis, and an estimation of the organic content for each layer separately. The method adopted for the mechanical analysis was the one advocated by Robinson (1922) which is recognised to give a more

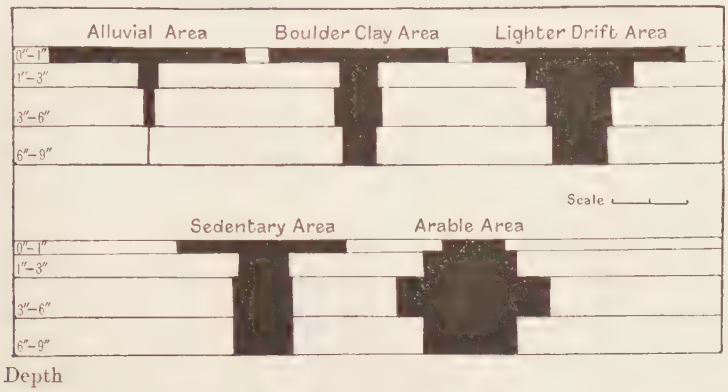

Fig. 3. Distribution in depth of the soil fauna in the five areas.
Scale, 1 div. = 200 individuals.

exact result than the ordinary agricultural sedimentation methods. A mechanical analysis of representative samples of the layers gave the following results.

Table IV.

Mechanical Analysis of the Soil of the Boulder Clay area at Different Depths.

	Depth in inches	
	1–3	3–6
Fine gravel	3·7	4·0
Coarse sand	5·4	4·6
Fine sand	10·7	10·4
Silt	12·0	13·75
Fine silt	38·0	39·5
Clay	14·5	13·5
Moisture	3·2	3·2
Loss on ignition ...	11·9	10·7

It will be noted that the mechanical composition and the organic content is apparently insufficient to account for the peculiar vertical distribution of the fauna. However, there is a distinct difference between these layers *in situ*. The upper layer (1–3 in.) presents an appearance of a dense mass of closely packed particles while the lower appears much more

porous. This compactness of the 1–3 in. layer may probably be the result of the interaction of the high clay and fine silt content with the pasture condition. The effect of rain and trampling by the larger grazing animals is, naturally, more marked nearer the surface than at lower levels. The Lighter Drift, being composed of larger sized particles, possesses a much lower degree of plasticity, resulting in higher percentage of the soil population in the 1–3 in. layer, compared with that of the corresponding layer in the Boulder Clay soil.

There is a distinct difference in the distribution in depth of the soil fauna of the Sedentary and Lighter Drift pastures (Fig. 3). This difference may be associated with at least two main factors. One is that the Sedentary soil contains such a high percentage of stones (Table II) that the conditions within the soil can be regarded as being more favourable for the deeper penetration of soil organisms than those of the Lighter Drift pasture. The other factor is that by reason of the predominant shallow-rooted plant species *Cynosurus cristatus* in the Sedentary pasture, the turf or surface covering is less thick, affording freer access of air into the lower layers and more equable conditions at greater depths.

In the arable area there was an increase of soil organisms with increase of depth in the upper six inches of soil, the depth to which arable land is usually cultivated (Fig. 3). This may be related to the loss of moisture, especially in the surface three inches, owing to progressive evaporation. The loose surface soil interrupts the capillary passage of moisture to the surface, and thus tends to a greater conservation in the deeper layers. Also the organic matter in an arable soil is more evenly distributed throughout the soil, and the latter being less compact, owing to the cultural operations, contains a higher percentage of free soil air than a similar soil of a permanent pasture. It therefore seems to be to the advantage of soil organisms in arable land to entrench deeply, so avoiding the consequences of sudden changes of weather conditions.

The tendency of modern investigations has been to show that soil organisms do not descend to greater depths during the winter months. The present data suggest—that in the lighter type of soils the organisms do descend to deeper levels but that in the heavier soil types the reverse is the case. This difference in seasonal behaviour of the fauna of different soil types in regard to vertical distribution has every appearance of being related to differences in the situation and mechanical composition of the soil, both of which in turn are associated with geological formation, and are themselves concerned in regulating the degree of moisture, aeration and temperature.

B. Components of Fauna in Relation to the Different Soil Types.

It seems that the laws governing plants in their relation to soils apply in the main to the soil fauna. In the Alluvial area one of the predominant

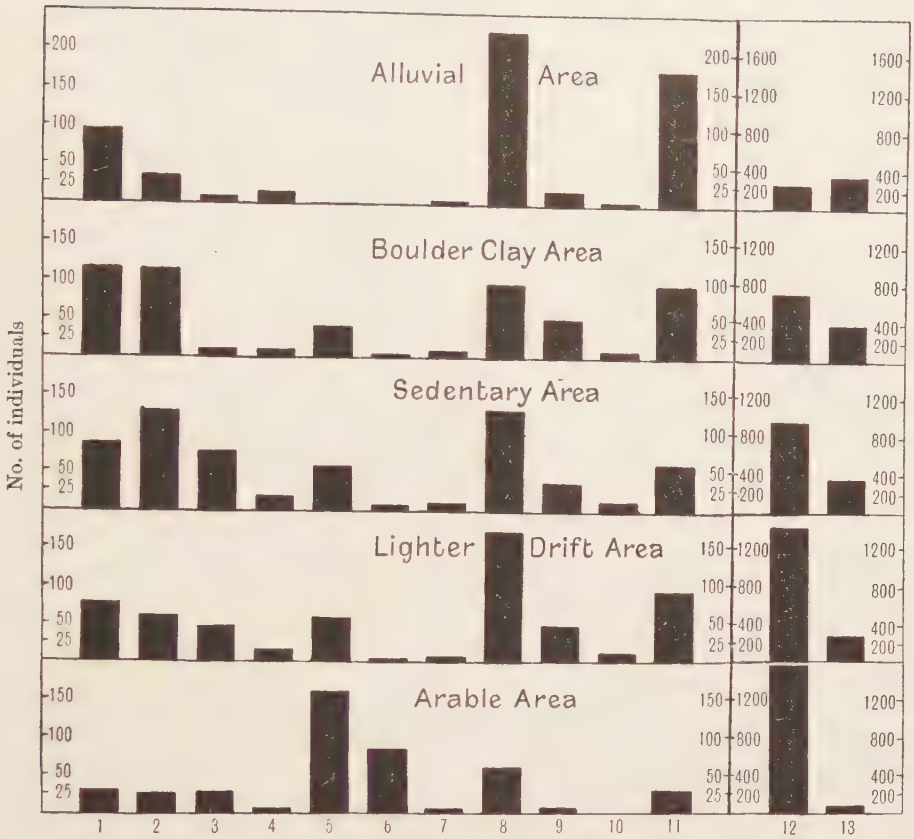

Fig. 4. Relative frequency of occurrence of individual groups.

1, Terricola; 2, Terricolae cocoons; 3, Nematoda; 4, Gastropoda; 5, Symphyla; 6, Myriapoda other than Symphyla; 7, Araneida; 8, Acarina; 9, Coleoptera; 10, Rhynchota; 11, Diptera; 12, Collembola; 13, Limicolae.

weeds was *Juncus effusus*, which is essentially a marshland species (Table I), and the majority of the Dipteran larvae also were aquatic or semi-aquatic forms.

It is noticeable (Fig. 4) that in the four pasture areas the Collembola, Oligochaeta (Limicolae) and Acarina were much the most abundantly

represented groups, and that the Oligochaeta (Terricolae), Diptera and Coleoptera were also fairly numerous in the four areas. Gastropoda, Rhynchota and Araneida were poorly represented. Symphyla and Nematoda showed a considerable difference in numbers, the former as well as Chilopoda and Diplopoda not being found in the Alluvial area.

Injurious insects occurred in all the grassland areas. The total numbers of Elaterid larvae recorded were 2, 14, 2, 3, for the Alluvial, Boulder Clay, Sedentary and Lighter Drift pastures respectively. The corresponding figures for Tipulidae larvae were 0, 3, 4 and 6. The larvae of *Athous haemorrhoidalis* were, except for single specimen, confined to the Boulder Clay, while the larvae of *Agriotes obscuris* occurred in all the areas.

It is noticeable that there is a marked difference in the fauna of the arable and pasture areas of the Lighter Drift. Cultivation seems to be associated with considerable increase in the number of Myriapoda and the Collembola, Onychiuridae spp. and with a reduction in the number of other groups, including Collembola other than Onychiuridae spp., represented in Fig. 4. Diplopoda contributed over 75 per cent. of the Myriapoda population (exclusive of Symphyla) in the arable area, and were represented by four species. Of the four species *Brachyiulus* (*Microbrachyiulus*) *pusillus* was best represented, forming about 56 per cent. of the total Diplopodan population. It will be noted that the Diplopoda were on the whole most numerous in the surface three inches, while the Chilopoda were more abundant at greater depths, where the soil was richest in fauna suitable for their food.

9. NOTE ON *OLIGOTROPHUS ALOPECURI* AND *APHIOCHAETAE BREVICOSTALIS*.

As many as 235 larvae of a Cecidomyid, *Oligotrophus alopecuri*, occurred in a sample examined from the Alluvial portion of Cae'r Efail. The larvae were of a crimson or pinkish colour and occurred gregariously near the base of *Alopecurus pratensis* grasses between the old sheath and the stem. Members of this species were not found parasitic on any other grasses. Their presence on the plant involved no serious effects except withering of the lower leaf. Before pupating the larvae seem to bore their way through the leaf sheath and pupate on the plant itself. The first adult appeared on March 20th under laboratory conditions, and has been doubtfully classified as *Oligotrophus alopecuri* Reut.

Another interesting case of parasitism was observed during the investigation. A number of specimens of the Phorid, *Aphiochaetae brevicostalis* Wood, were reared from a *Tipula* sp. larva. The latter when

obtained were alive. This parasitic Dipteran has only been recorded and bred before from dead snails; it may prove to be of economic significance.

10. SUMMARY.

1. Samples of soil were taken from four distinct soil types of a permanent pasture, and one of an arable land at the College Farm, Nantcellan Fawr, Aberystwyth. It is believed that the areas chosen are representative of their type in the district.

2. The pasture areas studied have been in pasture for at least forty years, while the arable area has been continuously cultivated, apart from an occasional rest in grass of from two to three years, for an equally long period.

3. Mechanical, chemical, botanical and faunal analyses were made.

4. The method of investigating the fauna consisted in taking samples of soil of standard size. The soil was removed in layers so that the approximate depth at which the insects and other invertebrates occurred, could be stated.

The soil of each layer, after a preliminary examination in the laboratory, was sieved in water. In order to deal as effectively as possible with the smaller organisms the residue left on each sieve was transferred to a shallow dish, and covered with water when the remaining smaller animals either floated on the surface of the water, or remained on the surface of the residue at the bottom of the dish.

5. The greatest number in the pasture areas, both of insects and other invertebrates, occurred in the surface inch layer, but some species were found in larger numbers at a greater depth. Thus the greatest number of Symphyla were found at a depth of six to nine inches and of *Tullbergia quadrispina* usually at a depth of three to six inches.

6. Some species, such as *Myrmica scabrinodes* were confined to the Sedentary area, and *Folsomia quadrioculata* to the Alluvial area, while other species occurred in all the areas. Collembola, *Onychiurus armatus*, while occurring in all the areas, were more common in the Lighter Drift areas.

7. The examination of cultivated land revealed a considerable increase in Symphyla, Diplopoda and some Collembola (*Tullbergia* sp.) and a reduction in the number of other Collembola, Oligochaeta, Acarina, Coleoptera and Diptera, compared with a pasture area of a similar soil type.

8. While qualitative difference may exist between the fauna of the several areas, the main differences concern rather the proportions in

which the various organisms occurred. There is decidedly less difference both qualitatively and quantitatively between the animal associations than there is between the plant associations.

9. An attempt was made to correlate the faunal data obtained in each area with the general environmental conditions. It was suggested that the differences in horizontal and vertical distribution were probably associated:

(i) Mainly with situation and mechanical composition of the soil, which in turn determines the degree of moisture, aeration and temperature at any given region within the soil.

(ii) With the nature of the flora, in that it affects the density and thickness of the surface turf and means of shelter and protection above ground as well as influencing the degree of evaporation.

(iii) With the depth at which particular food occurred especially to carnivorous animals, such as Chilopoda.

10. Injurious insects occurred in the four pasture areas. The larvae of Elateridae and Curculionidae were found in all but were scarce in the Alluvial. The larvae of Tipulidae were absent from the Alluvial, but were present in the other three.

11. Parasitism was infrequent, but a larvae of *Tipula* sp. was found attacked by *Aphiochaeta brevicostalis* Wood and *Alopecurus pratensis* by *Oligotrophus alopecuri* Reut. (?).

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INVESTIGATION ON *HETERODERA SCHACHTII* IN LANCASHIRE AND CHESHIRE

PART I. THE INFESTATION IN CERTAIN AREAS AS REVEALED BY CYST COUNTS; AN ESTIMATION OF THE ERRORS INVOLVED IN THE TECHNIQUE AND A CORRELATION WITH INTENSITY OF DISEASE

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(With 4 Text-figures.)

Introduction.

Although Kühn (3) as early as 1881 recognised that *Heterodera schachtii*, the beet eelworm, could occur on the roots of potato plants, it was not until 1913 that Zimmermann definitely recognised it as a serious parasite of the potato and, in 1920, published a paper (6) dealing with its occurrence and the damage which it produced on the potato crop in his own country. A few years later, Morgan (4) recorded the presence of a similar disease in Lincolnshire, but it has been recognised at many centres all over Britain.

As yet no satisfactory account of the life history of *Heterodera schachtii*, as it occurs on potatoes, has been published. Most investigators have been content to assume that the course of the life history of this strain on potatoes is identical with or very similar to that of the much better known strain on sugar beet, a very full description of the structure and development of which is given by Strubell (5).

Object of Investigation.

The failure of many potato crops in recent years has been attributed to "eelworm disease," but much doubt has been expressed as to the actual rôle of the eelworm in the disease. If there is a relationship between the eelworm infestation of the soil and plant disease, it is necessary to have a

satisfactory technique for measuring the infestation. The most obvious and direct method appears to be the counting of the encysted females, preferably in samples of soil collected during the winter months. The work reported in this paper was carried out in order to arrive at a suitable method of determining infestation by sampling and counting, with a computation of the errors involved. An attempt was then made to correlate differences in cyst counts with variations in intensity of disease in the previous potato crop.

Area Investigated.

Although, as subsequent pages will show, the occurrence of "eelworm" seems to be fairly widespread in Lancashire and Cheshire, attention was directed in the first instance to a few localities where potatoes are probably the most important crop in a short rotation and where the rotation could be lengthened only with difficulty. In the Mersey basin to the west of Manchester lies a fairly extensive area of peat. A considerable portion of this has been reclaimed by draining, burning the surface vegetation and cultivating. The resulting peat soil, frequently modified to a great extent by very heavy applications of marl and city refuse, is excellent for market garden produce and potatoes, and is farmed intensively rather than extensively. Usually potatoes are followed by oats and seeds, occasionally wheat comes into the rotation, but on the other hand potatoes frequently appear every second year, cabbage, celery, and so on, being taken up irregularly. Late frosts are sometimes troublesome and may damage the crops to the end of June so that late varieties, planted about the beginning of May, are always grown. A potato disease has been known to exist in this area since 1922 but was usually confined to comparatively small patches in the fields. In 1927 cysts of *Heterodera schachtii* were recognised as being associated with diseased plants and there was evidence that the trouble was reaching important dimensions, as fairly large tracts of ground yielded but a fraction of the normal crop.

In West Lancashire a second area of peat occurs, and, bordering on the peat proper, there are to be found soils which are perhaps best described as peat sands. Apparently, a thin layer of peat, together with heavy dressings of organic manures, has become incorporated with the first 15 inches or so of underlying sand (usually white) to give a dark grey soil with a sandy or sandy loam texture. On account of the sand substratum, which is usually at least three feet thick, these soils are well drained. In this area the farming is rather more extensive and varied than in the former, but potato growing is a most important source of

revenue to the farmer. In this area *Heterodera schachtii* was identified in 1923, and during the last two years "eelworm" disease has been observed in several localities.

Technique.

Field. Generally speaking, a strip about 5 yards wide, and running through the centre of a patch previously known to be affected with disease, was selected for examination. The strip was subdivided into lengths varying from 10 to 15 yards so that each plot sampled had an area of from 50 to 75 sq. yards. At least ten borings to plough depth were made at intervals over each plot, a soil auger being employed for the purpose. Except in two cases which will be discussed later, the borings were mixed to give a composite sample. This method was devised to get a representative sample of soil to plough depth and overcome the difficulty due to the fact that some centres had been ploughed whilst others remained unploughed at the time of sampling.

Laboratory. The composite samples were spread out in the laboratory and allowed to reach an air-dry condition. They were then broken up and the material passing through a 2 mm. sieve was employed in the examinations. For cyst counts, ten samples of 10 c.c. were taken, by the usual method of quartering, from each composite sample. The cysts were removed from the soil by the method described by Morgan (4). The sample of soil was placed in a Stohmann shaking bottle or a standard flask, and shaken with about 200 c.c. of water for 4 or 5 minutes. The flask was then filled up with water and allowed to stand until the cysts had floated with undecayed vegetable matter to the surface. The floating material was then thrown on to a filter paper and the cysts counted under a low power lens. A number of cysts adhere to stones and fragments greater than 2 mm. and are therefore lost in the above method. To estimate the loss incurred, the material greater than 2 mm. in diameter was examined in ten cases for peat soils, and in four cases for sandy soils. The greatest loss was 3·3 per cent. for a peat soil and 1·2 per cent. for a sandy soil, the respective averages being 2·5 per cent. and 0·9 per cent. As will be seen from the tables which follow, these figures, which were fairly constant for the two soil types, are scarcely worthy of serious consideration.

Sources of Error.

The counts made as described are liable to two important sources of error namely, (*a*) the field error due to taking ten borings to form a composite sample representative of a plot, and (*b*) the laboratory error due to

the sampling and counting of the sieved air dried soil. An attempt has been made to express those errors in the form of percentage standard error.

Laboratory error. Sufficient data are available to form a good estimate of the standard error due to laboratory sampling and counting, for over 90 samples, yielding about 900 counts, were carefully examined. Table I is typical of a series of samples and is set out at length to show the variation found in the individual counts of a sample and the method of arriving at the standard error of each mean.

Table I.

The cyst counts in a series of ten composite samples and the estimation of the laboratory standard error.

Sample 120	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*
	32	44	41	57	47	41	48	40	54	35
	34	51	42	48	42	36	50	52	28	25
	44	43	30	49	53	48	54	61	52	28
	31	44	29	42	50	36	48	49	41	37
	35	39	39	42	47	44	59	65	48	42
	31	39	39	49	57	44	68	59	40	30
	21	44	33	37	58	42	50	64	58	30
	40	42	35	52	45	30	51	55	42	29
	33	50	37	45	40	43	57	48	64	30
	32	49	50	52	52	34	46	40	43	27
$\bar{X}$	33·3	44·5	37·5	47·3	49·1	39·8	53·1	53·3	47·0	31·3
$S(X-\bar{X})^2$	328·0	162·0	348·0	312·0	325·0	181·0	399·0	748·0	874·0	240·0
σ^2	36·4	18·0	38·7	34·7	36·1	20·1	44·3	83·1	97·1	26·7
$\sigma^2/\sqrt{10}$	1·91	1·34	1·97	1·86	1·90	1·42	2·10	2·88	3·12	1·63
% error	5·73	3·02	5·25	3·94	3·87	3·56	3·96	5·41	6·63	5·22
χ^2	9·85	3·64	9·28	6·60	6·62	4·55	7·52	14·04	18·60	7·67

Average percentage error = 4·66.

$\bar{X}$ = the arithmetic mean of each set of ten individual counts.

If X = any one count, $(X-\bar{X})$ is the deviation from the mean.

$S(X-\bar{X})^2$ = sum of the squares of the deviations.

σ^2 = the variance = $\dfrac{S(X-\bar{X})^2}{n-1}$ for small samples, when n equals the number o counts (2).

σ = the standard deviation and $\sigma/\sqrt{10}$ = the standard error of the mean.

$\chi^2 = \dfrac{S(X-\bar{X})^2}{\bar{X}}$.

The results of every sample have been subjected to the same treatment to get an estimate of the standard error due to sampling and counting in the laboratory. For each series of plots, the percentage error has

been averaged[1] for those counts of more than ten cysts per 10 c.c. of soil. That limit has been chosen arbitrarily in order to exclude those samples taken from a strip running beyond an area actually infested and those samples in which the number of cysts was so small as to make the standard error quite abnormal. Table II gives a summary of the results.

Table II.

The percentage standard errors due to laboratory technique and the indices of dispersion.

Soil type	Series	Number of samples	Average standard error per cent.	Sn	χ^2
Peaty sand	23–32	10	7·34	90	93·11
	124 *A*–124 *E*	4	6·82	36	29·21
	66–75	3	6·44	27	30·92
	Weighted mean		7·06		
Peat	46–55	4	6·22	36	34·01
	113 *A*–113 *C*	3	7·89	27	42·44
	11–20	10	4·31	90	61·44
	111 *A*–111 *D*	4	9·02	36	40·82
	112 *A*–112 *D*	3	8·49	27	39·67
	6–10	2	5·49	18	18·83
	56–65	10	5·86	90	84·01
	120 *A*–120 *J*	10	4·66	90	88·37
	101–110	10	5·30	90	91·61
	Weighted mean		5·76		
	Total	73	—	657	654·44

The figures in Table II show that when sandy soils are considered the standard error due to laboratory technique is of the order of 7·1 per cent., whilst for the peat soils, which incidentally were found to have much greater counts, the error is in the neighbourhood of 5·8 per cent. That laboratory error appears to be large, but a more careful consideration of the position indicates that it is what might be expected. If a particle is taken from the soil, the chance of its being a cyst is very small indeed: if, therefore, samples consisting of large numbers of particles are taken at random from a bulk sample, the numbers of cysts in those samples should be distributed according to a Poisson series. An agreement of the results with the theoretical distribution affords a test of the suitability of the technique, and they have been submitted to an analysis similar to that employed by Fisher, Thornton and Mackenzie (1). For all Poisson series

[1] Since only an estimate of the error was desired the arithmetic mean has been taken.

the variance is numerically equal to the mean, and the index of dispersion, $\chi^2 = \frac{S(X - \bar{X})^2}{\bar{X}}$, is distributed in a known manner so that, for every value it assumes, there is a corresponding value P representing the probability that χ^2 will be exceeded by chance. In other words, it is possible to test the agreement between the results obtained and those expected. As a first approximation σ^2 was plotted against $\bar{X}$ and Fig. 1 shows that the majority of the points lie fairly closely to the line representing a true Poisson series. With a single sample of ten counts the range of variation of χ^2 is too great to be of much value, but the sum of any number of quantities χ^2 is distributed in the χ^2 distribution, and it is therefore at least possible to test if the variability from expectation is normal. The values of χ^2, calculated as in Table I for each sample of ten counts, have been summed for each series of samples, Table II. In this case Sn is equal to the sum of the various values of n for the separate samples, n being one less than the number of counts.

To test if the value 654·44 for χ^2 is normal for $n = 657$, use has been made of the fact that for such a large value for n, $\sqrt{2\chi^2}$ is approximately normally distributed about $\sqrt{2n-1}$ with unit standard deviation. In this case

$$\sqrt{2\chi^2} = 36{\cdot}18,$$
$$\sqrt{2n-1} = 36{\cdot}24,$$
$$\text{Difference} = -{\cdot}06.$$

The difference is much less than the standard deviation, so that the variability between parallel counts is quite normal.

Finally, values for χ^2 are set out in Table III at intervals alongside the expected values taken from a χ^2 table (2).

Table III.

Comparison of observed and expected distribution of χ^2.

χ^2	Expected	73 % expected m	Observed $m+x$	x^2/m
4·168	10	7·3	7	0·012
5·380	10	7·3	7	0·012
6·393	10	7·3	6	0·232
8·343	20	14·6	22	3·750
10·656	20	14·6	18	0·792
12·242	10	7·3	2	3·849
14·684	10	7·3	5	0·725
and over	10	7·3	6	0·232
Total	—	73·0	73	$\chi^2 = 9{\cdot}604$ $P = 0{\cdot}2$

The agreement is very good. By taking eight groups the probability of obtaining a worse fit by chance from normal data is about ·2, so that there is no significant deviation of the values from expectation. The analyses of the results seem to indicate, therefore, that most of the sets meet the conditions required by samples from the Poisson series and that, therefore, the technique was satisfactory and the mean value for each set of counts a reliable estimate of the number of cysts of *Heterodera schachtii* present in the soil sample. It follows that if the technique were perfect there would still be an inherent percentage standard error equal to $\sqrt{\frac{20{\cdot}44}{10}} \times \frac{100}{20{\cdot}44} = 6{\cdot}99$ for the peaty sands and $\sqrt{\frac{32{\cdot}18}{10}} \times \frac{100}{32{\cdot}18} = 5{\cdot}57$ for the peats, where 20·44 and 32·18 are the respective means when samples having counts less than 10 are omitted as previously. The values found compare very favourably with those calculated and may therefore be employed with some confidence as giving a measure of the laboratory error.

Field error. A large variation in the numbers of cysts from point to point on a plot is to be expected. To get figures for that variation in order to arrive at an estimate of the error involved in making a composite sample of ten borings, the following experiment was carried out. Ten samples were taken from each of the plots 124 *C* and H1, and examined separately. Ten counts were made for each sample. Table IV summarises the results.

The values 13·1 and 10·6 represent the percentage standard errors of the mean of a total of mn counts on n borings

$$= \sqrt{\frac{{\sigma_L}^2}{mn} + \frac{{\sigma_F}^2}{n}} \qquad \ldots\ldots(A),$$

where $m = n = 10$,

$\sigma_L/\sqrt{m}$ = the percentage standard deviation of the mean of m counts.

$\sigma_F/\sqrt{n}$ = the percentage standard deviation of the mean of n borings.

The value for $\sigma_F/\sqrt{n}$, representing the field error, has been calculated from equation (A).

Total error. The "total standard error" of the mean of m counts on a composite sample of n borings is then equal to $\sqrt{\frac{{\sigma_L}^2}{m} + \frac{{\sigma_F}^2}{n}} = 14{\cdot}7$ per cent. for the peaty sand and 11·7 per cent. for the peat.

The extent of those errors is due largely to the abnormal variations of samples 32 and 106 from the respective means. If those samples are

Table IV.

Variation in cyst content over two small plots and estimation of field errors.

Peaty sand plot 124 *C*			Peat plot 11*		
Sample	Mean of 10 counts (X)	$(X-\bar{X})^2$	Sample	Mean of 10 counts (X)	$(X-\bar{X})^2$
23	16·3	7·3	101	25·8	100·0
24	14·0	25·0	102	32·0	4·8
25	11·4	57·8	103	25·5	106·1
26	10·6	70·6	104	45·7	98·0
27	15·9	9·6	105	35·1	0·5
28	22·3	10·9	106	62·7	723·6
29	17·8	1·4	107	26·3	90·3
30	19·5	0·3	108	45·3	94·1
31	24·3	28·1	109	31·8	16·0
32	37·5	342·0	110	27·9	62·4
Total	189·6	553·0	—	358·1	1295·8
Mean $\bar{X}$ ...	18·96	—	—	35·8	—
Variance (σ^2)		61·4			143·9
Standard error of mean ($\sigma/\sqrt{10}$)		2·48			3·79
Percentage standard error ...		13·1			10·6
Percentage $\sigma_L/\sqrt{10}$		7·3			5·3
Percentage $\sigma_F/\sqrt{10}$		12·8			10·5
Percentage "total error" ...		14·7			11·7

* Samples 101–110 were taken 6 months later than composite sample 11.

excluded the "total standard errors" become respectively 11·1 per cent. and 9·1 per cent. The differences are considerable and serve to show how the large variation in infestation over a small plot may influence the results. It would be advisable to increase the number of borings taken to make the composite sample. For example, if the sample were obtained from 20 borings and 10 counts were made, the error of the mean would be reduced to about 9 per cent. for the peats. It is doubtful if increasing the number of borings beyond 20 would serve much useful purpose since a large increase in the size of the sample to be handled in the laboratory would make satisfactory manipulation difficult. As evidence of the fluctuations actually obtained, the following results are of some interest. Plot 124 *C* was sampled in the usual way, the mean of the ten counts being 19·7 compared with 18·96 for the ten samples 23–32, or 16·9 for the nine samples 23–31. Plot 46 overlapped plot 113 *B* and the respective means were 23·5 and 25·0. In both cases, therefore, the difference between duplicates was quite insignificant, being less than the standard error.

Taking the standard error as about 14 per cent. for the peaty sands

and 11 per cent. for the peats, it is now possible to review the results in greater detail.

Discussion of Results.

In order to simplify comparison and avoid unnecessary repetition all the results have been summarised and are presented in Table V. Each cyst count represents the arithmetic mean of ten counts; the samples comprise the following series. Series 23–32, to which reference has already been made, consists of ten samples from plot 124 *C*. The series 124 *A–E* lies in a strip through a small field which has been devoted for 8 years, with one break, to continuous cropping with potatoes. The soil is a peaty sand in type, containing about 10 per cent. of organic matter, and a heavy dressing of farmyard manure is given annually. Cysts of *Heterodera schachtii* were found on the roots of the potato plants in 1923 but there was no apparent disease of the shoots. Last season there was still some doubt as to whether there was any disease showing in the aerial parts of the plants, except in one corner of the plot 124 *C*. Taking the average standard deviation of one plot as 2·2 (14 per cent. of 15·8, the mean of the five plots) and the standard error between two

Table V.

Figures for cyst counts of soils examined.

No.	c.c.	No.	c.c.	No.	c.c.	No.	c.c.	No.	c.c.
23	16·3	124 *A*	17·0	11	46·7	111 *A*	12·5	6	29·7
24	14·0	124 *B*	14·8	12	41·2	111 *B*	10·6	—	—
25	11·4	124 *C*	19·7	13	30·5	111 *C*	20·1	10	42·0
26	10·6	124 *D*	18·6	14	35·0	111 *D*	13·9	—	—
27	15·9	124 *E*	9·0	15	32·4	—	—	56	45·7
28	22·3	—	—	16	32·3	112 *A*	21·7	57	33·8
29	17·8	46	23·5	17	27·9	112 *B*	19·7	58	21·4
30	19·5	47	26·1	18	30·1	112 *C*	16·0	59	23·9
31	24·3	48	24·9	19	44·9	112 *D*	6·6	60	22·5
32	37·5	49	7·4	20	40·2	—	—	61	23·3
—	—	50	19·0	—	—	120 *A*	33·3	62	25·5
66	8·4	51	7·6	101	25·8	120 *B*	44·5	63	24·6
67	3·8	52	8·1	102	32·0	120 *C*	37·5	64	20·0
68	27·9	53	1·9	103	25·5	120 *D*	47·3	65	18·8
69	40·8	54	0·3	104	45·7	120 *E*	49·1	—	—
70	19·0	55	0·2	105	35·1	120 *F*	39·8	—	—
71	4·3	—	—	106	62·7	120 *G*	53·1	—	—
72	0·6	113 *A*	21·0	107	26·3	120 *H*	53·3	—	—
73	0·1	113 *B*	25·0	108	45·3	120 *I*	47·0	—	—
74	0·1	113 *C*	29·2	109	31·8	120 *J*	31·3	—	—
75	0·0	—	—	110	27·9	—	—	—	—

c.c. represents number of cysts per 10 c.c. soil.

plots as $\sqrt{2} \times 2{\cdot}2 = 3{\cdot}1$, the only plot which may be regarded as differing significantly from any of the others is 124 *E*; the difference between 9 and the next but one highest count is 2·56 times the standard error of the difference between two plots. In other words, $P = {\cdot}01$ and the odds

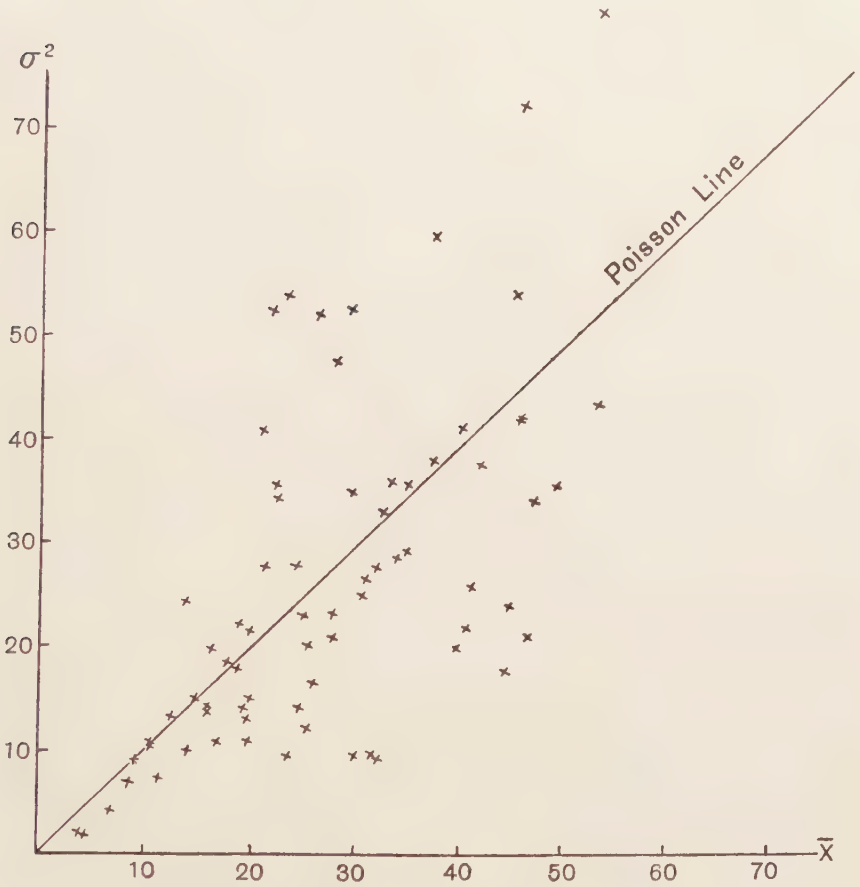

Fig. 1. Diagram representing association between $\bar{X}$ and σ^2 for cyst counts.

against that difference being exceeded as a fluctuation of sampling is about 99 to 1. The infestation of the other four plots may be said to be uniform, whilst the difference between 124 *B* and 124 *E* is barely significant.

Series 66–75 refers to a strip, 100 yards in length, through a field near Ormskirk, the soil being similar to that described above. In 1926

the potato crop was normal except for an isolated patch where the shoots were so diseased that they did not attain a height of 6 inches while the tubers were so small that they were not harvested. The disease had not previously been noted in that field. The usual crop rotation practised in the district is potatoes, wheat, oats, seeds, but this field was again planted with potatoes in 1927. The manuring was considerably altered and consisted of 2 cwt. each of ammonium sulphate, steamed boneflour and superphosphate per acre instead of the usual dressing of 20 tons of city refuse with 1 cwt. each of ammonium sulphate and steamed boneflour. The affected patch observed in 1926 bore, in 1927, a yield of tubers estimated at 60 per cent. normal and the diseased condition of the foliage was not very pronounced. The area of the patch had not increased. Plot 69 of the strip coincided roughly with that patch. There is no need to analyse the results further for there exists a significant increase in number of cysts to plot 69 and then a gradual decrease to nothing, the maximum infestation occurring on the affected patch (Fig. 2).

Series 46–55 consists of a strip, also 100 yards in length, running through a field on Barton Moss, the soil being decomposed peat modified by marling and city refuse, and overlying raw humus. On ploughing this type of soil for potatoes, it is customary to turn up one or two inches of the raw humus which gradually decomposes. The level of the moss land, partly for this reason and partly on account of continued drainage, has fallen considerably since cultivation was started. A 3-course rotation is practised on this farm, the potato crop being followed by oats and then seeds. The manuring for potatoes is usually about 30 tons of city refuse and 2 cwt. of phosphate. Lime is generally applied prior to cultivation for potatoes. There is no history of disease in this particular field prior to 1927 when a patch of dimensions 5 by 30 yards (113 *B*) occurred near one end. On this patch the amount of foliage of the potato plants was only about one-fifth of that in the immediate neighbourhood. As far as the aerial parts of the plants and yield of tubers were concerned, there was no apparent disease outside the patch, although cysts were found in large numbers on the roots of plants many yards distant. The accompanying sketch, Fig. 3, shows the position of the affected area and the two series 46–55 and 113 *A–C*. There is no significant difference between plots 113 *A*, 113 *B*, and 46–48; those, however, have figures significantly greater than all the other plots except 113 *C*. Plots 49, 51, 52 may be regarded as uniform but significantly less infected than plot 50, and significantly more infected than 53, 54, 55. There is, therefore, an undoubted general diminution of infestation away from the diseased area.

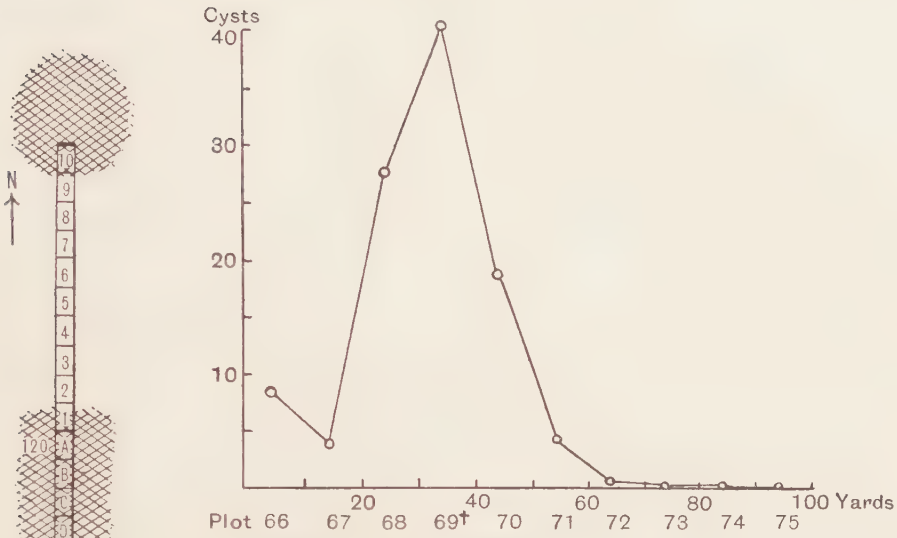

Fig. 2. Infestation through series 66–75: † affected area.

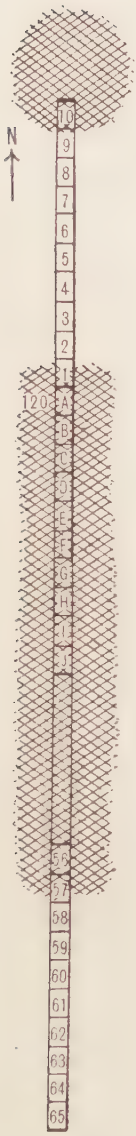

Fig. 4. Location of plots: shaded areas showed reduced yields.

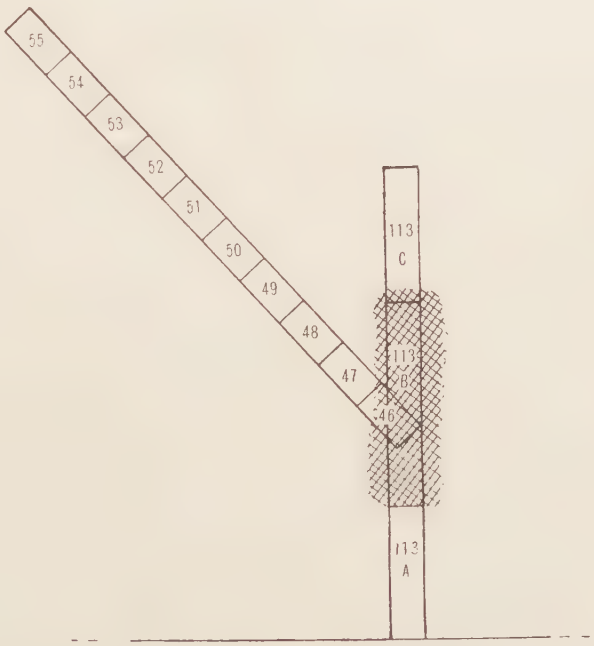

Fig. 3. Position of plots 113 *A–C*, 46–55: much affected area shaded.

The series 11–20 and 111 *A–D* are from a field on which the soil, rotation and manuring conditions are essentially the same as those in the last case considered except that lime is generally applied prior to sowing out with seeds. A diseased condition of the potato crop was observed in 1924; but it was not until 1927 that cysts of *H. schachtii* were recognised as being associated with the diseased plants. Three centres of disease were observed in 1927. Two of these areas were fairly extensive but the third was small. The disease on the third, however, was so intense that the crop was practically a failure and was harvested early. The series of samples 11–20 were taken from a strip of plots each 5 yards wide and 10 yards long, running through that area. From 11 to 15 the disease was bad, the worst place being in the locality of plot 11. Plots 16, 17, 18 were over an area on which there was no apparent disease. The records are indefinite regarding plots 19 and 20. Plots 111 *A–D* extend through one of the areas less affected. Nothing of a definite nature can be said regarding those series except that the cyst infestation is significantly greater in the series 11–20. In the series 11–20 the figure for plot 11 is greater than those for the six plots 13–18 by amounts which are quite significant. On the other hand, those six plots are essentially uniform in cyst content. In this series, therefore, there appears to be, as far as the information goes, some association between intensity of disease and cyst content. In the other case, 111 *A–D*, the disease was not very bad and was evenly distributed.

The series 112 *A–D* runs across a small field on Barton Moss, the soil conditions being similar to those in the last two cases. The farm is devoted mainly to market garden produce but potatoes are grown usually every third year. Heavy dressings of city refuse are applied and liming is carried out frequently. In 1926 disease was present to a slight extent on plot *A* but was absent from the others. In 1927 half of plot *A* was devoted to cabbages but the rest of the strip had potatoes. Disease was bad over plot *B* but gradually diminished through *C* and was very slight over *D*. The figures for plots *A* and *B* are not significantly different and the difference between plots *A* and *C* is just significant, being about twice the standard deviation between two plots. Plot *D* is certainly less infected. It would again appear, therefore, that there is an association between intensity of disease and cyst infestation.

The samples for the series 1–10, 56–65, and 120 *A–J* were taken from a strip running through a field on Carrington Moss. The soil, rotation and manuring are similar to those already mentioned for peat soils with the exception that lime is not applied. Disease had occurred in patches in

1922; in 1925, the next year for potatoes, the disease was very marked, several of the patches observed in 1922 having run together to form large oval-shaped areas, yielding less than four tons per acre of tubers (40 per cent. normal). The accompanying sketch, Fig. 4, indicates the principal features of those series. The disease was not quite uniform through the plots, and while small patches, where the crop was an entire failure, occurred irregularly, the disease gradually diminished north towards 120 *A* and the crop was normal about plots 5 and 6. Taking 56–65 into consideration first there is certainly a sudden drop in cyst count from plot 56 to plot 57 and from that to plot 58; the differences between 56 and 57, 57 and 58, are three times the standard error between two plots and are therefore significant. The plots 58–65 on the other hand may be regarded as uniform, the greatest difference being 6·7, which is less than twice the standard error. It is of interest to observe that an examination of 230 cysts from the above series showed that 31 per cent. were empty and black in colour. They may have been the product of the 1922 crop.

The infestation on plot 10 is significantly greater than that on plot 6; the results for the other plots of this series are not available. The series 120 *A–J* is most irregular, a fact which seems to fall into line with the observations on the disease. Since these observations were not very detailed, however, no purpose would be served by closer examinations of the cyst counts. It is noteworthy, however, that as in other cases, although cysts were invariably present in areas suffering from disease, they were also to be found in the vicinity of plants which seemed to be quite healthy.

Miscellaneous Samples.

From a stock of soil samples taken in the course of general advisory work, fourteen, from land used for potato growing, were selected at random. Cysts of *Heterodera schachtii* were found in seven of those samples and the counts are submitted (Table VI) merely to show that

Table VI.

Cyst counts of soils from different areas.

No.	Soil type	Locality	Cysts per 10 c.c.	*p*H
S 16	Sandy loam	Knutsford	3·6	5·56
S 47	Clay peat	Worsley	0·7	4·90
84	Peat	Halsall	2·7	4·94
95	Peaty sand	Bickerstaffe	9·1	4·76
96	Peaty sand	Bickerstaffe	0·4	6·22
128	Peaty sand	Bickerstaffe	0·8	6·83
130	Heavy loam	St Helens	3·5	7·88

the nematode is not confined to certain limited areas but appears to exist throughout the province.

Conclusions.

A study of the results as a whole indicates that there is a positive association of intensity of disease of the plants and cyst content of the soil in those cases where the disease has been observed recently. The figures for the two series 66–75 and 46–55 on different soil types are fairly conclusive in that respect. Where, however, the disease was noted several years ago that association is no longer always evident, for a moderately high cyst content may exist where there is no obvious disease. There would, therefore, appear to be some other factor, or factors, at work. A high susceptibility of the young plants to the effects of eelworm attack and a tendency for the majority of the larvae which hatch out later in the season to be attracted to the healthy and necessarily more mature plants, might be advanced as one explanation for the higher cyst numbers occurring outside an affected centre. Further, it is quite probable, as has often been suggested, that "eelworm disease" may be due to an association of the fungus *Rhizoctonia solani* with *Heterodera schachtii*.

The authors would like to express their thanks to Messrs I. S. Macdonald, H. W. Miles, J. Orr and E. Holmes Smith, of the Agricultural Advisory Department, for assistance in collecting the records on crop failure, locating diseased areas and making the soil samples, and to Mr L. Tippett for advice in the statistical treatment of the results.

Summary.

1. *Heterodera schachtii* has been identified in many parts of Lancashire and Cheshire where severe losses have resulted from failure of the potato crop.

2. A detailed study of the errors involved in the technique of a method for estimating the infestation has been made.

3. The degree of infestation, as determined by cyst counts, has been compared with records of crop failure.

4. The conclusions that have been drawn are to the effect that: (*a*) where "eelworm disease" has been noted recently there is a positive association of intensity of disease and cyst content of the soil; (*b*) where the disease was observed three or four years ago, disease and crop failure are always associated with a high cyst content of the soil, but there may be a moderately high infestation without apparent diminution of crop yield.

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INVESTIGATIONS ON *HETERODERA SCHACHTII* IN LANCASHIRE AND CHESHIRE

PART II. THE RELATIONSHIPS BETWEEN DEGREE OF INFESTATION AND HYGROSCOPIC MOISTURE, LOSS ON IGNITION AND *p*H VALUE OF THE SOIL

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(With 2 Text-figures.)

It has been shown (Part I) that an infestation of *Heterodera schachtii* may not be uniform over even a small area. It seemed desirable, therefore, to make an examination of certain soil characteristics to see if any relationship existed between the degree of infestation and the nature of the habitat. It was thought that the rate of reproduction of the nematode might be influenced by moisture and temperature conditions. Hence, attention was first directed to those physico-chemical properties which are directly related to soil moisture and temperature.

Moisture and Loss on Ignition.

Keen and Russell(3) have produced data which show qualitatively that the moisture variations in a soil are inversely related to the mean temperature; in other words, the soil warms as it dries and *vice versa*, and a rapid rise of temperature, in spring for example, does not occur until the soil has lost its excess of moisture. In another investigation on single value determinations, Keen and Coutts(4) have shown that a good correlation exists on the one hand between content of organic matter and the moisture at the "sticky point," and on the other hand, between clay content and the equilibrium moisture content at 50 per cent. humidity. The last mentioned is in turn closely related to "air dry moisture." The soils in the present investigation were either peaty sands or peats and it seemed, therefore, that the simplest method of obtaining comparative figures for field conditions of moisture and temperature was to determine the moisture content and the loss on ignition of the air-dried soil.

All the samples concerned were made in the course of a few days,

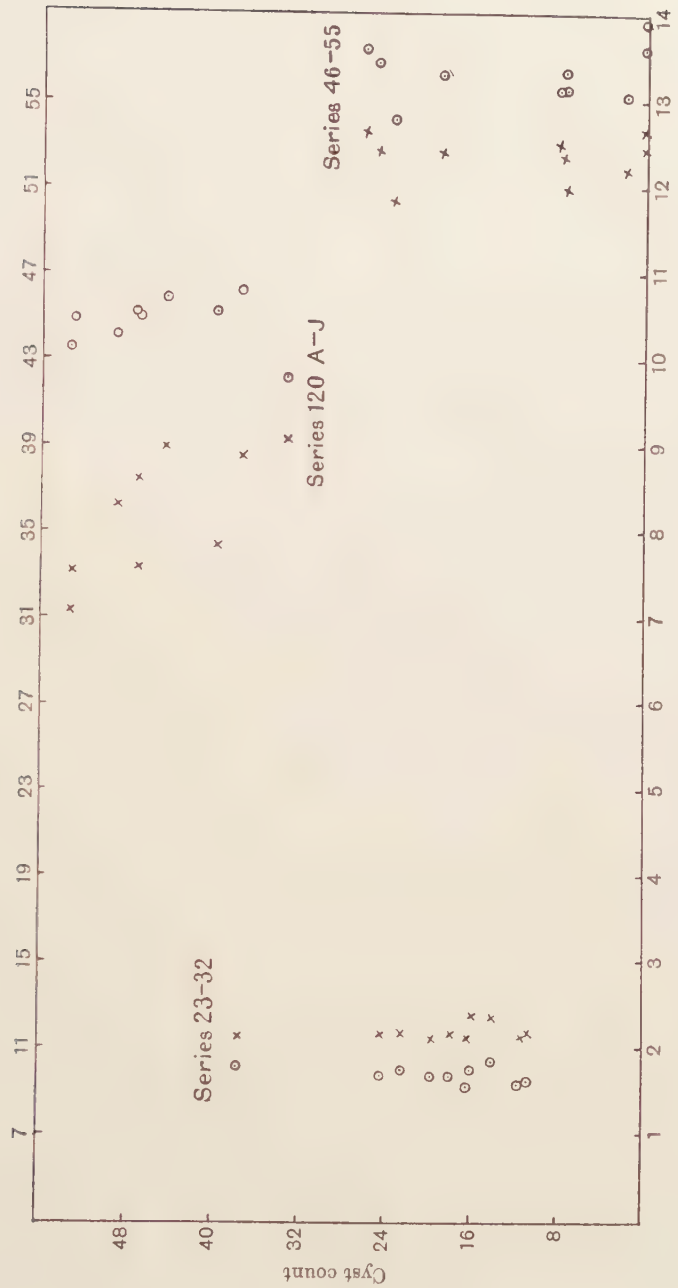

Fig. 1. Diagram showing relationship between infestation of *H. schachtii* and moisture, and loss on ignition for three series of soil samples.

while the samples of any one series were taken in one day. The air-dried and sieved (2 mm.) soil was employed. The usual procedure was to heat the soil to a temperature of 100–105° C. for about five hours, weigh and then ignite to constant weight. The conditions were identical for each series and the determinations were made in duplicate. Values were obtained for three series in each of which there was a wide and significant variation in cyst content.

The values for both moisture and loss on ignition are, however, quite scattered and uncorrelated with the cyst counts (Fig. 1). It is almost certain that characteristics arising from excessive dryness on the one hand, or bad drainage and excessive moisture on the other hand, would have been revealed by those measurements. Since those series were typical of the different field conditions met with in the course of the investigation it seemed useless to pursue this line of study further.

Acidity.

Another soil property upon which a vast amount of work has been carried out, and the determination of which provides a useful figure in the consideration of acidity and base exchange problems, is the *p*H value. It gives a measure of the hydrogen ion concentration of a soil or of, what is usually determined, an aqueous suspension of the soil. An investigation on the relationship between *p*H and cyst counts was carried out by Peters (5), who made the measurements colorimetrically on water extracts of the soil. The bulk of his observations refer to one experimental field where the *p*H ranged from about 6 to 6·7, and the cyst content from about 3 to 80 per 10 c.c. of soil. From those results he concluded that there was "an indubitable correlation between *p*H and cyst-concentration" but that a set of miscellaneous samples confirmed that correlation only in part, the reverse sometimes being found.

The *p*H of the soil samples was determined electrometrically by means of the quinhydrone electrode (1, 2). The technique was as follows. About 10 gm. of the sieved soil was shaken vigorously for one minute with about 25 c.c. water and a few decigrammes of quinhydrone. After standing for a few minutes, connection was made with a standard quinhydrone electrode and the potential difference observed. The instrument employed permitted the readings to be made to one millivolt (roughly equivalent to 0·02 *p*H). The standard error was about $\pm$ 0·02 and the results have been given to two places of decimals, although it is realised that such accuracy is liable to misconception in an investigation of this nature.

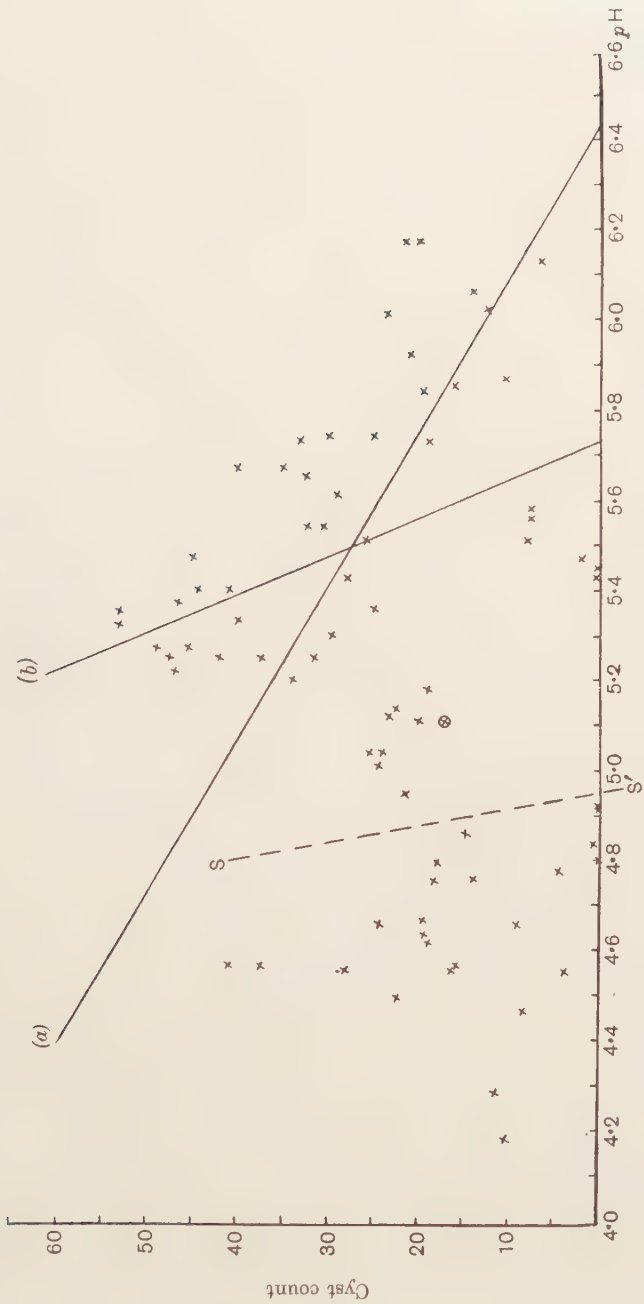

Fig. 2. Diagram showing association between cyst count and pH of soils.

Table I.

Cyst counts and pH values of soil samples.

Series 23–32		Series 124 *A*–124 *E*		Series 66–75		Series 46–55		Series 113 *A*–113 *C*		Series 112 *A*–112 *D*	
c.c.	*p*H	c.c.	*p*H	c.c.	*p*H	c.c.	*p*H	c.c.	*p*H	c.c.	*p*H
16·3	4·56	17·0	5·11	8·4	4·47	23·5	6·01	21·0	5·92	21·7	6·17
14·0	4·76	14·8	4·86	3·8	4·56	26·1	5·51	25·0	5·74	19·7	5·84
11·4	4·29	19·7	4·67	27·9	4·56	24·9	5·36	29·2	5·61	16·0	5·85
10·6	4·19	18·6	4·76	40·8	4·57	7·4	5·56			6·6	6·13
15·9	4·57	9·0	4·66	19·0	4·62	19·0	5·73				
22·3	4·50			4·3	4·78	7·6	5·58				
17·8	4·80			0·6	4·84	8·1	5·51				
19·5	4·64			0·1	4·92	1·9	5·47				
24·3	4·66			0·1	4·92	0·3	5·43				
37·5	4·57			none	4·80	0·2	5·45				

Series 11–20		Series 111 *A*–111 *D*		Series 6, 10		Series 56–65		Series 120 *A*–120 *J*	
c.c.	*p*H	c.c.	*p*H	c.c.	*p*H	c.c.	*p*H	c.c.	*p*H
46·7	5·37	12·5	6·02	29·7	5·30	45·7	5·27	33·3	5·73
41·2	5·40	10·6	5·87	42·0	5·25	33·8	5·20	44·5	5·40
30·5	5·54	20·1	6·17			21·4	4·95	37·5	5·25
35·0	5·67	13·9	6·06			23·9	5·04	47·3	5·25
32·4	5·65					22·5	5·14	49·1	5·27
32·3	5·54					23·3	5·12	39·8	5·33
27·9	5·43					25·5	5·04	53·1	5·35
30·1	5·74					24·6	5·01	53·3	5·32
44·9	5·47					20·0	5·11	47·0	5·22
40·2	5·67					18·8	5·18	31·3	5·25

The first three series are peaty sands, the remainder are peats.

For the purpose of a preliminary examination the two variables, *p*H and cyst count, were plotted in the form of a dot diagram (Fig. 2). The figures for *p*H range from about 4·2 to 6·2, and those for cyst count from 0 to about 53 per 10 c.c. soil. At first there appeared to exist no simple association unless it were that the maximum concentration occurred in the neighbourhood of *p*H 5·3 and that less cysts were found on either side of that point. The picture, however, is considerably altered when the two soil types—peat and peaty sand—are regarded separately. By a fortunate chance all but one of the peaty sands are more acid than the most acid of the peat soils and the broken line *SS′* effects a simple division of the two groups: a circle has been drawn round the unique point of the smaller group, occurring beyond the line *SS′*. A consideration of the points representing the larger group of peat soils to the right of *SS′*

shows that there appears to be a general trend indicating a negative correlation between *p*H and cyst count. Mention has already been made of the totally different characters of the two soil types, and the figures of Table II emphasise those differences in physical properties as exemplified by apparent specific gravity. It was decided, therefore, to analyse the results for the two groups separately.

It was further considered advisable to regard all the pairs of observations as one large sample from an area over which the occurrence of *H. schachtii* is widespread. The *p*H through a strip 100 yards long on a field usually fluctuates to a certain extent owing to a variety of causes, but the range is rather short and ten pairs of observations at most, too limited in this case to secure a satisfactory coefficient of correlation. Taking the 53 pairs of observations as a whole, therefore, the figures for *p*H vary from 4·95 to 6·17, those for cyst count from 0 to 53·3 per 10 c.c. soil.

If X = any cyst count and Y the corresponding *p*H value,

x = the deviation of any cyst count from the mean $\bar{X}$ (27·25),

y = the deviation of any *p*H value from the mean $\bar{Y}$ (5·50),

then r, the coefficient of correlation $= \Sigma xy/\sqrt{\Sigma x^2 \Sigma y^2}$.

In this case $r = -0{\cdot}504$.

The probability that such a correlation would have been obtained from any random comparison is less than 0·01, so that the correlation is undoubtedly significant. The coefficients of regression (6)

$$b_1 = r\frac{\sigma_x}{\sigma_y},\quad b_2 = r\frac{\sigma_y}{\sigma_x},$$

where σ_x and σ_y, the standard deviations of cyst counts and *p*H, are respectively 13·86 and 0·2361, have been calculated:

$$b_1 = -29{\cdot}59,\quad b_2 = -0{\cdot}008592.$$

The regression equations are therefore $x = -29{\cdot}59\,y$,

$$y = -0{\cdot}00859\,x,$$

or replacing x by $(X - 27{\cdot}25)$ and y by $(Y - 5{\cdot}50)$

$$X = 190{\cdot}05 - 29{\cdot}59\,Y \quad (a),$$

$$Y = 5{\cdot}7342 - 0{\cdot}00859\,X \quad (b).$$

The dot diagram has been completed by inserting the lines representing the equations (*a*) and (*b*). An examination of the diagram reveals that the correlation coefficient is reduced by the group of values for the observations 58–65 (see p. 336, Part 1) all below *p*H 5·20 and with comparatively low cyst counts. They appear to belong to another population.

There is little doubt, therefore, that some correlation, worthy of further investigation, exists between cyst count and *p*H, but that there are other factors at play.

When the twenty-five pairs of observations for the sandy soils are subjected to the same treatment, the coefficient of correlation is found to be − 0·176 which is certainly not significant.

Table II.

Apparent specific gravities of two soil types.

Type	Peaty sand		Peat soil				
Sample	Composite 124 *B*		111 *A*	112 *A*	113 *A*	120 *A*	120 *I*
Apparent spec. grav.	1·20	1·23	0·55	0·61	0·54	0·61	0·54

Summary.

1. Soil samples taken for the purpose of measuring the infestation of *Heterodera schachtii* have been further examined for any association between cyst concentration and those physico-chemical properties relating to the moisture, temperature and acidity of the soil.

2. The hygroscopic moisture and loss on ignition have been determined for three typical series of ten samples having significant variations in cyst counts. There is no simple association of the observations within any of the series. It appears improbable, therefore, that the rate of reproduction of *Heterodera schachtii* is influenced to a marked degree by the normal variations in the physical condition of the soil.

3. The *p*H of seventy-eight samples has been measured electrometrically. When the samples are divided into two groups according to soil type, there is found to be a significant negative correlation between *p*H and cyst count for fifty-three peat soils. There is not, however, a significant correlation in the case of the other twenty-five sandy soils.

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REVIEWS

Physiology and Biochemistry of Bacteria. Vol. I. Growth Phases; Composition and Biophysical Chemistry of Bacteria and their Environment, and Energetics. By R. E. BUCHANAN and E. I. FULMER. Pp. xi + 516; 78 Figs. London: Baillière, Tindall & Cox. 1928. $ 7.50.

Bacteriology has for long been merely the handmaiden of Pathology and Industry and a Science of Bacteriology can at present hardly be said to exist. A vast amount of empirical data has been accumulated but this is scattered in almost heart-breaking fashion through journals devoted to agriculture, general biology, medicine, chemistry, physics and various industrial applications and the need is perhaps peculiarly urgent for the compilation and systematisation of this material.

Chapter I of the present volume, consisting of a little over two pages, is devoted to a consideration of the scope of physiological bacteriology. It gives what is really the keynote of the work and is summarised in the statement that 'physiological bacteriology includes all the applications of mathematics, physiology, chemistry, and physics to the problems of the life and growth of micro-organisms." In support of this comprehensive claim the authors, in later chapters, make wide ranging excursions into territories which are not usually regarded as pertaining to bacteriology. Their wide net sweeps up not only bacteria, but fungi, slime molds, protozoa and even algae and the reader is led into mazes of physics, chemistry and mathematics which are apt to confuse the essential issues. The embracing character of the work may be gauged from a skeleton outline of its contents.

Chapter II deals with the growth phases and growth rates of micro-organisms in culture. Following a review of the various methods, direct and indirect, of estimating numbers and amounts of growth in culture, there is a discussion of the actual growth phases of various kinds of micro-organism and a consideration of growth itself as an autocatakinetic phenomenon. In relation to this field of study the authors sound a much needed warning against "interpreting causal relationships on the basis of curves plotted or the equations which describe them."

In Chapter III a discussion of microscopic and macroscopic methods of analysing the chemical composition of the cells of micro-organisms is followed by a lengthy consideration of the actual data of such analyses.

Chapter IV, containing 234 pages, is really a general treatise on physical-chemistry and in it are considered at length the nature of true solutions and of colloidal solutions and the colloidal state, and more briefly, such questions as refractivity, specific gravity, diffusion and conductivity. In discussing electricity as a tool in measurement the authors emphasise a point of view that might perhaps be more seriously considered by many teaching-schools of biology, viz. that "No biologist can be considered as equipped with a full kit of tools for the exploration of physiological process who is not conversant with the fundamentals, not only of electricity in general, but more especially of the behaviour of the cell, tissue, or tissue fluid toward the electric current."

In Chapter V are considered the energy relationships, growth and movement of micro-organisms. After brief reference to the units of force, work and power, the chapter is mainly devoted to a discussion of the types and sources of energy for micro-organisms and the various ends—synthesis, heat, light and movement—to which they are utilised.

The volume closes with 30 pages of "literature cited," author and subject indices and an index to names of micro-organisms in the text.

Volume II, which has not yet appeared, will be concerned with the influence of environmental conditions on microbial development, with changes produced by bacteria and with the mechanism of such changes.

It will be obvious that the work is planned on rather an epic scale and one must admire the authors' courage in setting out on such a task and be grateful for what they have already given us. One may be accused of looking a gift horse in the mouth but one cannot help feeling that the book might possibly have been more useful and successful if the authors had been content with a lesser ambition. After all, there are within easy reach excellent text-books of physiology, physics, chemistry, physical chemistry and mathematics and there seems little reason why this much needed volume on *The Physiology and Biochemistry of Bacteria* should be ballasted with so many of the fundamental teachings of these subjects. No one denies their importance in the nascent science of bacteriology, but their detailed exposition seems out of place as an integral part of a treatise of this nature and the resultant clogging of the more direct issues tends to induce a state of mental indigestion on the reader's part. On the other hand, it is undoubtedly convenient to have gathered together in a single treatise the relevant portions of so many diverse sciences and as the volume is "essentially a combination and revision of the lectures on physiology of bacteria and on biophysical chemistry given at the Iowa State College to students of bacteriology", one assumes that in practice this convenience has been found by the authors and their students to be so great as to outweigh other considerations.

As a book of reference the work is of a very considerable value. It is clearly and simply written and it incorporates into a logical scheme a great mass of data many of which would otherwise not easily be accessible. The standpoint of the authors is fair and judicious and where necessary both sides of a case are presented. There can be no doubt that the volume will fulfil the hope that it "may call forcibly to the attention of students the wide gaps that exist in our knowledge of the physiology of micro-organisms, and stimulate additional research in this field." It is perhaps characteristic of the viewpoint throughout that the work ends with the sentence "An explanation is not forthcoming!"

WILLIAM B. BRIERLEY.

Life in Inland Waters: with especial reference to animals. By KATHLEEN E. CARPENTER. With an Introduction by JULIAN S. HUXLEY. Pp. xviii + 267. London: Sidgwick and Jackson, Ltd. 1928. 12*s.* net.

The necessity for the provision in this country of facilities for the investigation of problems of fresh-water biology is being increasingly recognised and we have rather suddenly awakened to the fact that in the scientific study of our inland waters we lag considerably behind many other European countries. The general position to-day is, in fact, little different from that pictured by Kofoid in 1910 in his treatise on *The Biological Stations of Europe*. For many years well equipped limnological institutes have been in active existence in Russia, Germany, Austria, Sweden, Denmark, Belgium and other countries, whilst in England our only centre has been a small private laboratory established in 1901 by Mr Eustace Gurney on Sutton Broad. English investigations have been carried out by the Wests, the Pearsalls and other individuals mostly working from a University centre and carrying with them into the field such facilities as they could provide. More recently the Ministry of Agriculture and Fisheries have conducted investigations into the ecology of streams and considerable extension of this work is imminent.

The subject was brought to the front by Professor Fritsch who, in his Presidential Address to Section "K" of the British Association in 1927, emphasised the urgent need for the establishment of an active and well equipped fresh-water biological station in Great Britain. A further step was taken the following year when at Glasgow, Sections "D" and "K" held a joint discussion on the subject which resulted in the appointment of a small Committee to consider the means to be adopted for the

establishment of such a station. The Committee has just issued a report and it is greatly to be hoped that its recommendations will lead to the establishment of the suggested station in the English Lake District.

Meanwhile, by the publication of her admirable book, *Life in Inland Waters*, Dr Carpenter has most opportunely provided a text which should do much to help forward this very necessary development. The book is no dry-as-dust academic compilation but an interestingly written survey of the animal ecology of fresh waters and it has caught the very spirit of that fascination of "pond-life" which in our teens lured so many of us into biological ways.

Following an introductory chapter on the general characteristics of the fauna of inland waters, Chapter II deals with the components of the fauna and their general activities and interrelationships, and Chapter III with their relationships to the chemical and physical factors of the habitat. Chapter IV, dealing with the reproduction of fresh-water animals, is one of the least satisfactory portions of the book, but this is almost inevitable, for the author can only touch the surface of so thrilling and formidable a subject in the few pages allowable and this she has done extremely well. Chapter V is a tantalisingly brief consideration of the geographical distribution and dispersal of fresh-water animals and of the influence of the great ice age in Europe. The next four chapters deal with the special features of particular kinds of habitat and show how far these special features influence the life which characterises them. In two of these chapters we are taken from head streams and highland brooks along the minnow reaches and finally arrive at the lower reaches and estuarine waters. In the following two chapters are discussed the biology of lakes and of small or peculiar water bodies such as marsh and moorland waters, cold and hot springs, subterranean waters, etc. The last and perhaps most interesting chapter deals with the biology of inland waters in relation to human life and considers among other questions the vexed and urgent problems of the pollution of rivers by town drainage and industrial wastes.

The chapters are headed with delightfully apt quotations from Isaac Walton, that most lovable father of fresh-water biology, and terminated by very useful bibliographies that cover an unusually wide range of literature. There are good subject and author indices and the book is excellently illustrated by twelve plates and 94 text figures. The volume is one of the series of text-book of Animal Biology edited by Professor Julian Huxley, its format is very pleasant and as modern prices go it is good value for the money.

A volume of this size dealing with so comprehensive a subject must obviously be a little superficial, and yet on reading the book one is left rather with the impression that most problems of any importance have been at least indicated. Further, in spite of its size and interest the book contains an astonishing amount of data. Breathing new life into a subject that academic minds and laboratory researches have almost killed, this is a book that one would like to put into the hands of all undergraduate students of biology. There are few of us who, even now, do not like to dabble surreptitiously in "pond-life", and undergraduate students for whom "pond-life" is legitimate will find in Dr Carpenter's book the guide they need.

WILLIAM B. BRIERLEY.

The Scientific Principles of Plant Protection. By HUBERT MARTIN, with a Foreword by Sir DANIEL HALL. 316 pages. London: Edward Arnold. 1928. 21*s*.

A cordial welcome may be offered to this book, which should prove a valuable contribution to a subject of growing importance. Sir Daniel Hall remarks in his foreword: "There is a general theory of plant medicine or plant hygiene and Mr Martin's book is the first introduction to it which has appeared in English." It is certainly true that most previous works have treated the subject of "Plant Protection" primarily from the botanical or zoological aspects, and it is all to the good, therefore, that a

chemist, such as Mr Martin, should intervene in a field so largely occupied by entomologists or mycologists, and summarise the facts bearing upon "Plant Protection" from a different standpoint.

Mr Martin's method of dealing with his matter is to divide it into sections and subsections—*e.g.* **Plant Resistance**, **Insecticides**, and Contact Insecticides—and then, after some introductory remarks to each section, to quote or summarise the writings of the various investigators who have contributed information bearing upon the subject under discussion. From the chemical standpoint the literature appears to have been examined minutely: upwards of some 800 references are given to the works of nearly as many writers, and this alone will render the book most useful as a work of reference. Detailed criticism would be out of place because the author is giving not his own conclusions but those of other writers, and where so many investigators are concerned, it is inevitable that the articles quoted should be of unequal merit, a point, however, against which the junior reader might perhaps be warned. A few misstatements, or misinterpretations, will be noted (*e.g.* p. 4, that nematodes are "true worms with ringed bodies," and p. 245, that the Brown Tail Moth was introduced into the U.S.A. from England) but in the first edition of so comprehensive a compilation occasional mistakes are almost inevitable, and in the present case they do not detract from the usefulness of the whole. One suggestion may perhaps be made, and it is that the title is a little misleading. From the chemical aspect the book is much more than a statement of scientific principles, while from the biological side the title suggests fuller discussion of certain subjects than is actually afforded them. "Plant Protection from the Chemical Standpoint" would adequately describe at least 80 per cent. of the book, and would perhaps give the purchaser a better idea of what to expect. Subject and author indices are provided, and in so far as they have been tested appear to be satisfactory. The author is to be congratulated on having produced a book which will be of value to all concerned with "Plant Protection."

J. C. F. FRYER.

Spraying, Dusting and Fumigating of Plants. By A. Freeman Mason. 539 pages, 237 Figs. New York: The MacMillan Co. 1928. 21*s*.

This book is the most recent of the series of Rural Manuals edited by L. H. Bailey, a series which needs no introduction to English readers. The present volume resembles its predecessors in being practical and comprehensive, well printed and adequately illustrated: in fact, it is typical of American books of its class, and the opportunity may be taken of expressing admiration for the gift possessed by the American writer of rendering the results of scientific investigation available to the "practical man," although it must be admitted that this task is facilitated by the readiness of the American reader to digest a fair amount of technical matter if a commercial advantage can be secured.

In regard to Mr Mason's book, it may first be pointed out that whereas the title adequately describes the first half of the book, the second half—comprising something like 250 pages—is better indicated by the sub-title "a popular Handbook on Crop Protection," since it comprises a popular account of the more important American plant pests and diseases, with measures for dealing with them, not only by spraying, dusting, or fumigation, but by other means as well. This latter half of the book is necessarily of less interest than the first to the English reader, who for information as to American pests and diseases will rather refer to such works as Hesler and Whetzel's *Manual of Fruit Diseases*, or the similar work on Fruit Insects by Slingerland and Crosby: even so, it may be suggested that in a subsequent edition the scientific names of the various pests and diseases dealt with might be inserted, an omission seldom noted in an American work.

Turning to the sections of more general interest, Chapters I–V give some account of the history of spraying, the principles underlying spraying practices, and of the

insecticides and fungicides in general use. These chapters form a useful and convenient summary of the modern American viewpoint on these matters. It is a little odd, however, to find the attractiveness of geraniol to the Japanese Beetle dealt with on p. 26 under "Repellents," and the statements on pyrethrum on p. 66 are hardly in accord with the most recent work on the subject. English apple growers will read with envy on p. 50 that the American Apple Capsid can be controlled by the use of a nicotine spray containing only about 0·025 per cent. pure nicotine—half the strength required in spraying against the English Apple Capsid (*Plesiocoris rugicollis*).

Chapters VI to IX, dealing with "Selecting the Spraying Machine," "Qualifications of Spray Machinery," the "Central Stationary Spraying Plant," and the "Art of Spraying," form decidedly the most interesting part of the book, and should be read by all who are concerned with spraying whether from the research or commercial points of view. Specially worth attention are the remarks of the author upon the need for sufficient power in spraying apparatus. The smallest engine-driven sprayer which he considers of value is one with a single cylinder pump worked by a 1½–2 h.p. engine, with an average output of 3–4 gallons per minute at 250 lbs. pressure, and he emphasises that this will only supply one spray rod with two disc nozzles or one spray gun with a small orifice in the disc. This apparatus he considers useful for a 5-acre orchard. For spraying a 25-acre plantation he advocates a 3-cylinder pump and an engine of from 3½–6 h.p., and even so he gives a warning that unless the machine is at its best it will not satisfactorily supply more than one gun with a large aperture. This insistence on adequate power as the first essential in spraying machinery occurs throughout the chapters concerned, and is specially mentioned as it is well worth English attention. Of almost equal interest is the need for apparatus which will do the work sufficiently quickly—a need which is strongly emphasised by the author. He believes a satisfactory spraying equipment should be able to cover the whole area to be sprayed in four days, because on an average there are not more than four days fit for spraying in any week. In England this estimate would often prove too optimistic, and therefore the need for quick work is all the more imperative. Chapter VIII on the "Central Stationary Plant" shows that this method of dealing with the spraying problem, a method in which England is the pioneer, is now attracting considerably more attention in the U.S.A. Finally, reference may be made to Chapter X—"Dusts and Dusting," the author's considered opinion on this debatable subject being that "The day for unequivocal recommendations of dusting is not yet at hand but indications are that it will soon dawn. Until then the orchardist and gardener must consider the liquid sprays the standard materials." In this chapter some reference is made to dusting machinery, but it is relatively slight as compared with the descriptions of spraying apparatus given in the earlier chapters: in a subsequent edition a full discussion of the mechanics of dusting apparatus would be welcome.

On the whole the book, although written for the American "orchardist" and "truck crop" grower, has much in it of value to the English reader, and it is to be commended not only to the notice of the practical grower but also to that of the scientific investigator who might well devote more attention to the mechanical and engineering problems involved in pest control.

J. C. F. FRYER.

Praktische Einführung in die Morphologie der Insekten. By E. HANDSCHIN. Pp. viii + 112 + atlas of 23 plates. Berlin: Gebrüder Borntraeger. 1928. (Sammlung naturwissenschaftlicher Praktika, Bd. 16.) 11 R.M. cloth bd.

Notwithstanding the numerous text-books dealing with diverse aspects of entomology, both theoretical and applied, that have appeared during the last few years they leave the laboratory training of the student only scantily provided for. Practical

guides to the dissection and study of insects are so few and far between that Schoenichen's "Praktikum der Insektenkunde" (2nd edition) and Comstock and Needham's "Elements of Insect Anatomy" (10th edition) are the only works that can be recalled as being in any way comparable with the new book by Dr Handschin. In his *Praktische Einführung*, Dr Handschin has provided a type of manual that is particularly welcome and can be recommended as a thoroughly sound and reliable introduction to the elements of insect morphology. Throughout its pages he interprets structure in relation to function and having mastered the course thus planned for him, the student should be sufficiently equipped to understand the significance of the chief modifications that insects undergo. He should, for example, be able to deduce how a given insect lives, how it obtains its food and what kind of food it is dependent upon.

Dr Handschin's method of treatment is to divide the book into chapters, each dealing with a separate region of the insect body, and he utilises various types of insects in illustration of the diverse modifications the organ or region in question may undergo. The introductory chapter is concerned with (*a*) methods of preserving, fixing and mounting specimens; (*b*) a list of the principal works on general entomology useful to the student; and (*c*) an enumeration of the different species of insects required in order to follow the full course laid down. The succeeding chapters deal as follows: (I) with the chitinous skeleton; (II) the head; (III) the head appendages; (IV) the thorax; (V) the abdomen; (VI) the endoskeleton; (VII) auditory and chordontonal organs; and (VIII) the spiracles. Most of the chapters are further subdivided into sections or lessons and as an example of Dr Handschin's scheme of instructions section B—the mouth-parts, of Chapter III may be selected The section is preceded by a list of papers deemed useful to the student in his work: the list is short enough to avoid confusing a beginner and includes some of the important papers written in English and continental languages. There follows an enumeration of various common insects necessary for the student to have at hand in order to carry out the prescribed work on mouth-parts. The introductory type adopted is *Periplaneta*, which is used to explain the general plan of the insect mouth-parts and as an example of an insect with omnivorous feeding habits. The carnivorous type, as illustrated by *Cicindela* follows next, and the adaptions to a flesh-eating habit are clearly stressed. The herbivorous type is exemplified in the cockchafer and then there follows a short discussion of certain more special types of trophi as is seen in Collembola, *Dytiscus*, larvae, Odonata, etc. The student is next introduced to the nectar-sucking types of mouth-parts, Lepidoptera being utilised to illustrate the evolution of the haustellum, and various common Hymenoptera to demonstrate the part played by the ligula in sucking and "licking." The highly specialised piercing mouth-parts are considered under two categories. Firstly the blood-sucking type, and here the student is required to study a Culicid, followed by a Tabanid and finally *Stomoxys* and *Glossina*. Secondly the plant-sucking type which is conveniently illustrated by *Pentatoma* or *Graphosoma*. The sixth type of mouth-parts is the highly evolved suctorial and licking arrangement seen in the proboscis of various Diptera, *Musca* or *Calliphora* providing the necessary material.

As a further guide to the prescribed course an atlas of 23 plates is provided in a pocket at the end of the book. Here the student will find exceptionally clear figures illustrating practically every feature in insect structure that is dealt with in the text. The features which they portray are all taken from common and easily obtainable insects, most of which the student can collect for himself in almost any good locality.

By way of constructive criticism we make the suggestion that in the event of a second edition of the book being called for, as we think will happen, the author would be well advised to consider the inclusion of certain features of internal anatomy. Chapters on the respiratory, digestive and reproductive systems and their chief modifications would be valuable and not unduly enlarge the book. There is happily very little by way of adverse criticism that calls for mention. Typographical errors are few and these mostly have been noted and listed by the author on p. viii. On p. 35 Cantharididae is evidently meant for Cantharidae while throughout the book *Stomoxys* is spelled *Stomoxis* and *Lymantria* is spelled *Limantria* except on p. 9.

These, however, are very minor blemishes in a thoroughly sound elementary handbook.

A. D. IMMS.

The Problems of Applied Entomology. By Robert A. Wardle. Manchester University Press. Biological Series, No. V. 1928. Pp. xii + 587; frontispiece and 31 illustrations. 30*s.* net.

The author of this book, in conjunction with the late P. J. Buckle, published a work of a very similar character in 1923, entitled *Principles of Insect Control*. The latter volume was the first ever written dealing in a comprehensive manner with the multifarious aspects of the subject of insect control, and it was well received both in this country and in America. The present volume is to be regarded as supplementary to its predecessor and, to a large extent, only takes cognisance of work that has appeared since 1922: it is a considerably longer book with the subject matter arranged on a somewhat different scheme.

Part I, which comprises 247 pages, is devoted to General Problems, and under this heading the many different methods and factors that exercise a controlling influence on noxious insects are discussed. In these pages the reader will find a clear account of some of the most recent additions to knowledge of that wide range of subjects. Mention may be made of Uvarov's important theory of migratory and non-migratory phases in locusts; the immensely important subject of virus disease transmission; the insect control of weeds; chemotropism; biological control by parasites and predators; and an extended discussion on insecticides.

Part II, comprising 271 pages, discusses "Area Problems", and this section of the book breaks new ground, in that the author here gives a concise account of the problems applied entomologists have to face in different regions of the globe, the major pests that prevail there, and the "local" controlling measures in force. It brings together much scattered information not easy of access, and has evidently been the result of industrious search through a very large number of departmental bulletins, annual reports and circulars. The two concluding chapters of the section are headed "Locality Disinfection" and "Locality Protection." The expression locality is used in a somewhat vague implication since it may refer to the soil around the roots of a particular plant, or to a bale of cotton, a particular field or even to an extensive tract of country. By "Disinfection" is meant the destruction of noxious insects, and in the chapter "Locality Disinfection" three main problems are considered, viz. the destruction of insects affecting animals or plants (*a*) before the latter leave the locality or area wherein they have been reared, and (*b*) before or immediately after their admission into a new area, or at least before they become widely dispersed, while (*c*) discusses the eradication from a restricted locality of an introduced insect of known potentiality. Sections (*a*) and (*b*) deal with schemes which lie outside the more usual methods of insect control, in that they aim at the absolute eradication of the insects present, instead of reducing an infestation to a degree compatible with successful practice. Section (*c*) also differs from ordinary practice in that it does not take cognisance of control measures in relation to the market value of the product infested. It only considers the question of eradicating a dangerous insect, irrespective of cost. The chapter on "Locality Protection" is largely concerned with such legislative measures as embargoes and quarantines.

Part III (67 pages) is occupied with a classified bibliography, an index of authors referred to and a subject index: the bibliography is not intended to be complete, but it lists the representative economic literature of the past seven years, and judicious selection seems to have been exercised.

A book which embraces so wide a field usually presents some features wherein a reviewer may find himself not in complete agreement with the author. In the volume before us insecticides come in for a very large share of treatment: one would have liked to have seen a few of these pages devoted to a more extended discussion of the

growing subject of virus diseases and their insect transmission, which are so much in the minds of plant pathologists to-day. As a further suggestion, the section on biological control would repay extending. This method is attracting attention in almost every country of the world, but we are as yet only on the very fringe of the possibilities attending its application and the pitfalls are many. A fuller discussion of the principles involved, the possible causes of success and failure in specific cases, and some account of the technique of mass rearing of parasites would be a welcome improvement. These few points we hope may receive consideration in the event of a third volume of similar scope being contemplated.

Books of the type of the present one are becoming more and more necessary in all subjects, like applied entomology, whose advances are accompanied by such an enormous output of diverse kinds of literature. It is of the greatest assistance to the investigator, the teacher and the advanced student to have access to a work which takes stock of the present day position of the subject concerned. This fact Prof. Wardle has evidently recognised to the fullest degree and we congratulate him upon the production of a well written, well arranged and, it might be added, almost indispensable volume.

The publishers are to be complimented on the printing and general "get up" of the book, but it is a matter of disappointment that they have found it necessary to fix its price at so relatively high a figure.

A. D. IMMS.

REPORT OF THE COUNCIL OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS FOR THE YEAR 1928

During 1928 the Association has met on eight occasions. At five of these, various subjects of interest were brought before the Association by members and visitors to whom it is greatly indebted. The subjects included Marine Biology, Forest Products Research, Biology of Wood Wasps, Physiology of Plant Disease, and Preservation of Timber. At the Annual General Meeting the Presidential Address was given on Legislation in England against Diseases and Pests of Plants, which has been published in Vol. xv, No. 2 of the *Annals* of the Association.

In May, the Association had the privilege of visiting the Royal Botanic Gardens at Kew by kind invitation of the Director, and the summer meeting was held at Bristol and Long Ashton Research Station by courtesy of Professor Barker, the Director.

The first Annual Dinner was held on January 25th.

The attendance at meetings has varied from 39 to 57, with an average of 42. The proportion of members to visitors has been about 10 per cent.

During the year the Association has lost one of its valued members by the death of Dr W. G. Smith.

Two Honorary Members, Professor August Chevalier of Paris and Professor Filippo Silvestri of Naples were elected.

Thirty-three new members have been elected during the year; there have been two resignations and the Association now numbers 283 Honorary and Ordinary Members.

In view of the increase in sales of the *Annals of Applied Biology* it has been necessary to increase the number of copies printed by 100.

During the past year the Association has again enjoyed the hospitality of the Botany Department of the Imperial College of Science and Technology for their meetings. The Council feel sure that the Association will approve of recording its grateful thanks for this privilege.

Papers read to the Association during the year 1928:

Jan. 20th. Presidential Address: "Legislation in England against Diseases and Pests of Plants."

Feb. 24th. Mr F. A. Pantin: "The Work of the Plymouth Marine Laboratory."

March 23rd. Mr R. S. Pearson and Mr J. F. Martley: "On the Work of the Forest Products Research Laboratory."

Oct. 26th. Mr R. N. Chrystal on "Sirex and its Parasites" and Mr H. St J. Cartwright on "A fungus symbiont associated with *Sirex cyaneus*."

Nov. 23rd. Mr R. H. Stoughton on "The Relation of Environmental Conditions to Angular Leaf Spot Disease of Cotton." Dr W. F. Bewley on "The Effect of Environmental Factors on Diseases under Glass," and Mr T. Small on "Temperature and Humidity in Relation to *Cladosporium fulvum*."

Dec. 14th. Professor Percy Groom: "The Antiseptic Preservation of Wood."

REPORT OF THE HON. TREASURER FOR THE YEAR 1928

During the year 1928 current subscriptions received amounted to £272. 16*s.* 0*d.*, an increase of £22. 6*s.* 4*d.* over the previous year. Arrears of subscriptions amounting to £25. 0*s.* 0*d.* were paid while subscriptions considered good, but as yet unpaid, totalled £25. 10*s.* 0*d.*; the latter amount compares very favourably with the corresponding figure of £37. 15*s.* 0*d.* for 1927. The working expenses of the Association have been considerably less than those of the previous year which is entirely due to a reduction of £252. 19*s.* 3*d.* in the publishers' account for the *Annals of Applied Biology*, owing to very material increases in sales of back volumes and parts and of reprints of special articles. After net receipts for sales had been deducted the sum of £175. 16*s.* 5*d.* only was required to meet the publishers' charges. It is satisfactory to note that we close the year with a satisfactory cash balance while the incurred liabilities amount to £418. 12*s.* 4*d.* of which £388. 7*s.* 2*d.* is represented by the publishers' net charges for the cost of production of Vol. xv of the *Annals of Applied Biology*. The Association also has a reserve fund in Savings Certificates now amounting to £531. 5*s.* 0*d.*

A. D. IMMS,
Hon. Treasurer.

THE ASSOCIATION OF ECONOMIC BIOLOGISTS

Dr INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31ST DECEMBER 1928 *Cr*

EXPENDITURE	£	s.	d.	£	s.	d.	INCOME	£	s.	d.	£	s.	d.
To Annals of Applied Biology:							*By* Subscriptions:						
Estimated Value of Stock at 1 Jan. 1928	55	3	6				Arrears	25	0	0			
Expenditure during 1928	395	4	8				Entrance Fees	8	7	6			
	450	8	2				Current	272	16	0			
Less: Estimated Value of Stock at 31 Dec. 1928	62	10	8								306	3	6
				387	17	6	*By* Contributions to cost of papers in *Annals of Applied Biology*				103	19	6
To Printing and Stationery				13	4	5	*By* Interest on National Savings Certificates and Bank Deposit				54	5	6
To Postage and Cheque Stamps				7	1	11							
To Sundry Out-of-Pocket Expenses of Secretaries and Treasurer				14	13	2							
To Audit Fee Reserve				4	4	0							
To Balance, being Excess of Income over Expenditure				17	7	6							
				£444	8	6					£444	8	6

BALANCE SHEET, 31 DECEMBER 1928.

LIABILITIES AND SURPLUS	£	s.	d.	£	s.	d.	ASSETS	£	s.	d.	£	s.	d.
Sundry Creditors:							Cash:						
The Cambridge University Press	388	7	2				At Bank on Current Account	131	14	5			
Secretaries	6	16	2				At Bank on Deposit Account	455	0	0			
Audit Fee Reserve	4	4	0								586	14	5
				399	7	4	Debtors for Subscriptions 2 years or less in arrear and considered good				25	10	0
Subscriptions paid in advance				19	5	0	500 National Savings Certificates				531	5	0
Excess of Assets over Liabilities:							Stock of *Annals of Applied Biology*, at estimated value				62	10	8
As Balance Sheet of 31 Dec. 1927	770	0	3										
Add.: Balance of Income and Expenditure Account for 1928	17	7	6										
				787	7	9							
				£1206	0	1					£1206	0	1

A. D. IMMS, *Honorary Treasurer.*

We certify that the foregoing Accounts are properly drawn up in accordance with the books, vouchers and documents produced to us, and, in our opinion, the Balance Sheet exhibits a true and correct view of the state of the affairs of the Association.

W. T. ROWLINSON & CO.
Incorporated Accountants.
Auditors.

LUTON, *February* 5, 1929.